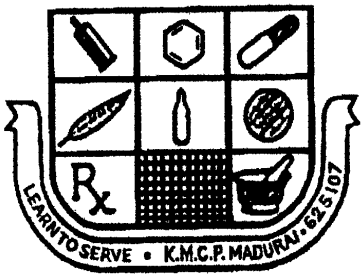
NEWER ANALYTICAL METHODS FOR THE DETERMINATION OF ROPINIROLE HYDROCHLORIDE IN BULK DRUG AND ITS FORMULATION

Dissertation Submitted in partial fulfillment of the requirement for the award of the degree of

MASTER OF PHARMACY

Of

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY, CHENNAI



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CERTIFICATE

This is to certify that the project entitled "**Newer Analytical Methods for the Determination of Ropinirole Hydrochloride in Bulk Drug and its Formulations" by JOTHIBASU K *(Reg. No. 26101722*)** in partial fulfillment of the degree of Master of Pharmacy in Pharmaceutical Analysis under The Tamil Nadu Dr. M.G.R. Medical University, Chennai, done at **K. M. College of Pharmacy, Madurai - 625107,** is a bonafide work carried out by him under my guidance and supervision during the academic year 2011-2012. The dissertation partially or fully has not been submitted for any other degree or diploma of this university or other universities.

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ABBREVIATION

%	-	Percentage
µg	-	Micro gram
µl	-	Micro litre
µm	-	Micro metre
ACN	-	Acetonitrile
Amt	-	Amount
ATC	-	Anatomical Therapeutic Chemical Classification
C_{18}	-	Octa Decyl Silane column
CAS	-	Chemical Abstracts Service
Cm	-	Centimetre
CZE	-	Capillary Zone Electrophoresis
HPLC	-	High Performance Liquid Chromatography
HPTLC	-	High Performance Thin Layer Chromatography
Hr	-	Hour
IUPAC	-	International Union of Pure and Applied Chemistry
KH_2PO_4	-	Potassium Dihydrogen Phosphate
LC	-	Liquid Chromatography
LC-MS	-	Liquid Chromatography-Mass Spectrophotometry
LOD	-	Limit of Detection

LOQ	-	Limit of Quantitation
mg	-	Milli gram
min	-	Minute
ml	-	Millilitre
mm	-	Milli metre
nm	-	Nano metre
ODS	-	Octa Decyl Silane Column
RPLC	-	Reverse Phase Liquid Chromatography
RSD	-	Standard Deviation
SD	-	Standard deviation
SEM	-	Standard Error Mean
Std	-	Standard
Tab	-	Tablet
TLC	-	Thin Layer Chromatography
UPLC	-	Ultra Performance Liquid Chromatography
λmax	-	Absorption maximum

1. INTRODUCTION

1.1 A General Approach to Analytical Chemistry

Analytical Chemistry has been defined in many ways. One of the most widely accredited definitions is that of the Working Party on Analytical Chemistry of the Federation of European Chemical Societies: "*Analytical Chemistry is a scientific discipline that develops and applies methods, instruments and strategies to obtain information on the composition and nature of matter in space and time.*" [1]

A complementary definition has recently been issued, according to which "*Analytical Chemistry is a metrological discipline that develops, optimizes and applies measurement process intended to derive quality bio-chemical or chemical information of global and partial type from natural and artificial objects or systems in order to solve analytical problems derived from information need*."

A trend has recently emerged for the systemic use of metrology in chemistry in order to explain the main field of action of analytical chemistry, namely the performance of bio-chemical or chemical measurements based on standards with a view to establishing comparisons in order to produce qualitative, quantitative and structural results.

1.2 Aims and Objectives of Analytical Chemistry

The intrinsic aim is the attainment of metrological quality, i.e. ensuring full constancy between the analytical results delivered and the actual value of the measured parameters; in metrological terms, this translates into producing highly traceable results subject to very little uncertainty.

The extrinsic aim is solving the analytical problems derived from the bio-chemical or chemical information needs posed by a variety of clients (e.g. private companies, social agents and research centers) or in other words providing client satisfaction.

The main objective of analytical chemistry is to obtain as much bio-chemical or chemical information and of as high a quality as possible from objects and systems by using as little material, time and human resources as possible and with minimal costs and risks. [1]

1.3 Classification of Analytical Chemistry

Based on the type of bio-chemical or chemical information delivered

- ***Qualitative Analysis*** - deals with the identification of elements, ions or compounds present in a sample.[2]
- ***Quantitative Analysis*** - deals with the determination of how much of one or more constituents are present in any compound or mixture of compounds.[3]

Based on the analytical technique used in the analytical process

- ***Classical Analysis (Wet Analysis)***
  - Acid-base titration
  - Non-aqueous titration
  - Argentimetric titration
  - Complexometric titration
  - Redox titration
  - Iodometric titration
  - Diazotization titration [4]

- ***Instrumental Analysis***

Spectroscopic Techniques

- Ultraviolet and visible spectrophotometry
- Fluorescence spectrophotometry
- Phosphorescence spectrophotometry
- Atomic emission spectrometry
- Atomic absorption spectrometry
- Infrared spectrophotometry
- Raman Spectroscopy
- X-ray spectroscopy
- Nuclear magnetic resonance spectroscopy
- Electron spin resonance spectroscopy [5]

Electrochemical techniques

- Potentiometric techniques
- Voltametric techniques
- Coulometric techniques
- Amperometric techniques
- Electrogravimetric techniques
- Conductance techniques [6]

Separation techniques

- High performance liquid chromatography
- Gas chromatography
- High performance thin layer chromatography
- Paper chromatography
- Thin layer chromatography
- Super critical fluid chromatography
- Capillary Electrophoresis
- Capillary Electro chromatography

Hyphenated techniques

- Gas Chromatography - Mass spectrometry (GC-MS)
- Gas Chromatography - Fourier Transform Infrared Detection (GC-FTIR)
- Gas Chromatography - Atomic Emission Detection (GC-AED)
- Liquid Chromatography - Mass Spectrometry (LC-MS)
- Liquid Chromatography - Fourier Transform Infrared Detection (LC-FTIR)
- Liquid Chromatography - Nuclear Magnetic Resonance Detection (LC-NMR)

Miscellaneous techniques

- Thermal Analysis
- Radio chemical methods [6]

1.4 ULTRAVIOLET AND VISIBLE ABSORPTION SPECTROPHOTOMETRY

Ultraviolet and visible absorption spectrophotometry is the measurement of the absorption of monochromatic radiation by solutions of chemical substances in the range of 185 nm to 380 nm and 380 nm to 780 nm of the spectrum respectively. The magnitude of the absorption of a solution is expressed in terms of the absorbance (A) defined as the logarithm to base 10 of the reciprocal of transmittance (T) for monochromatic radiation:

$$\mathbf{A = \log_{10} (I_o / I)}$$

Where I_o is the intensity of the incident radiation and I is the intensity of the transmitted radiation. The absorbance depends on the concentration of the absorbing substance in the solution and the thickness of the absorbing layer taken for measurement. For convenience of reference and for ease in calculations, the specific absorbance of a 1 per cent w/v solution is adopted in Pharmacopoeia for several substances unless otherwise indicated and it refers to the absorbance of a 1 per cent w/v solution in a 1 cm cell and measured at a defined wavelength. It is evaluated by the expression

$$\mathbf{A\ (1\ per\ cent,\ 1\ cm) = A /}\boldsymbol{cl},$$

Where *c* is the concentration of the absorbing substance expressed as percentage w/v and *l* is the thickness of the absorbing layer in cm. The value of A (1 per cent, 1 cm) at a particular wavelength in a given solvent is a property of the absorbing substance. Unless otherwise stated, the absorbance should be measured at the prescribed wavelength using a path length of 1 cm and at 24º to 26º. Unless otherwise stated, the measurements are carried out with reference to the same solvent or the same mixture of solvents.

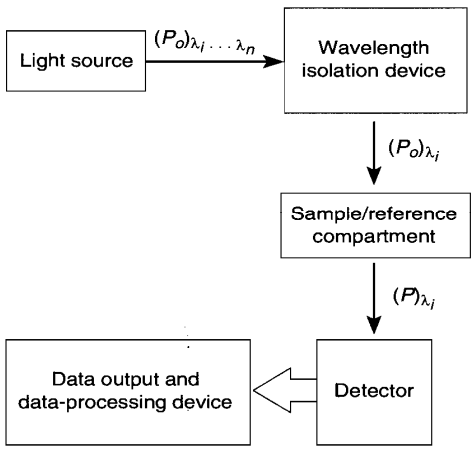
Apparatus

A spectrophotometer suitable for measuring in the ultraviolet and visible ranges of the spectrum consists of an optical system capable of producing monochromatic light in the range of 200 nm to 800 nm. The two empty cells used for the solutions under examination and the reference liquid must have the same spectral characteristics. Where double-beam-recording instruments are used, the solvent cell is placed in the reference beam. [7]

Instrument Components

Figure no. 1 depicts a block diagram of the essential components of a typical UV/Vis instrument. Some instruments position the sample/reference compartment before the wavelength isolation device. This arrangement permits significantly greater intensity of incident light falling on the sample and reference but may result in photo degradation of some thermally sensitive samples, possibly creating baseline problems with high fluorescent samples.

Figure no. 1 Block diagram of typical instrument for making UV/Vis absorption measurements



Light source

- Incandescent tungsten filament lamp
- Quartz – Iodine lamp
- Xenon arc lamp
- Hydrogen or deuterium arc discharge lamp

Wavelength Isolation Device

The purpose of the wavelength isolation device is to separate the many wavelengths of light coming from the continuum produced by the light source and isolate the particular wavelength of interest.

Spectrophotometer monochromators are using either diffraction grating or prisms as dispersive devices. Diffraction gratings are classified as either classically ruled or holographic gratings. Prism monochromators are another popular wavelength isolation device for spectrophotometers. There are many geometric designs of prisms, but the Cornu (60˚- 60˚- 60˚) and the Liittrow (30˚- 60˚- 90˚) prisms are most widely used and may be arranged in a variety of configuration.

Sample and reference compartments

Only double-beam instruments have a reference compartment. The double-beam instrument is usually configured so that monochromatic light from the wavelength isolation system is divided in by means of an optical beam splitter and passed through both the sample and reference compartments continuously or rapidly alternating between the compartments continuously or rapidly alternating between the compartments. This allows compensation for small variations in the light source and permits continuous blank corrections. The high stability of modern light sources coupled with rapid measurement system allows many high-quality instruments to incorporate a single-beam optical system without significantly sacrificing measurement stability and without the additional expense of the double-beam optics system. [8]

Detectors

For the accurate determination of substances by spectrophotometric techniques, precise determinations of the light intensities are necessary. Photoelectric detectors are most frequently used for this purpose. They must be employed in such a way that they give response linearity proportional to the light input and they must suffer from drift or fatigue.

- Barrier-layer cells
- Phototubes
- Photomultiplier tubes
- Photo diodes
- Thermocouples
- Bolometer
- Thermistors
- Golay detector [9]

Data Output and Data-Processing Device

Signal output devices can be as simple as an analog absorbance or transmittance meter where the data are read, recorded and processed by the operator. Some systems use logic circuitry to provide digital readouts in transmittance, absorbance or concentration. Most modern spectrophotometers now incorporate microprocessors for control and monitoring of instrument operation. These systems commonly provide an interface to a computer system along with software to control instrument operations, data collection and data processing.[8]

1.5 LIQUID CHROMATOGRAPHY

Liquid chromatography (LC) is a method of chromatographic separation based on the difference in the distribution of species between two non-miscible phases, in which the mobile phase is a liquid which percolates through a stationary phase contained in a column.[1]

Liquid chromatography is mainly based on mechanisms of adsorption in liquid-solid chromatography, mass distribution in normal phase liquid chromatography (NPLC) and reversed-phase chromatography (RPLC), ionic in ion-exchange chromatography (IEC), size exclusion or stereo chemical interaction in size-exclusion chromatography (SEC) and affinity in affinity chromatography. [10]

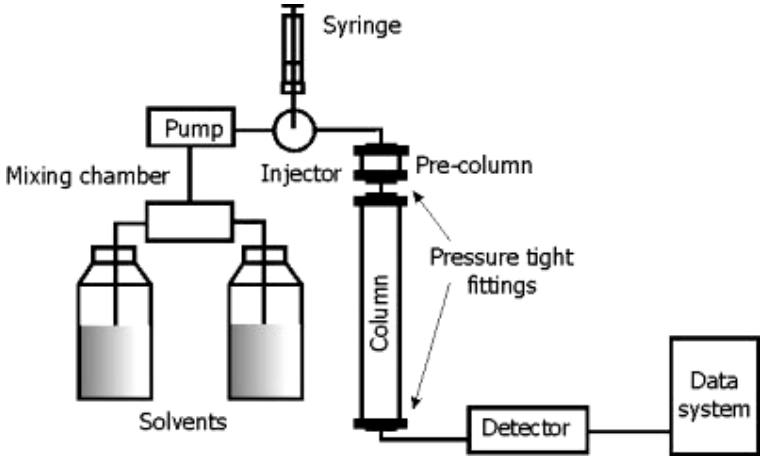
Table no: 1 Classification of liquid chromatographic techniques

Technique	Main separation mechanism	Stationary phase	Mobile phase
Adsorption chromatography	Adsorption	Polar material such as silica or alumina	Non-polar solvents
NPLC	Distribution/adsorption	Polar material	Non-polar solvents
RPLC	Distribution	Non-polar material	Polar solvents
IEC	Ion-Exchange	Charged solid phase material	Typical aqueous buffer solutions
SEC	Size exclusion	Porous solid phase material	Organic or aqueous solvents

Instrumentation

The modules of *HPLC unit* are illustrated in figure no. 2. It consists of a pump unit, solvent reservoirs, an injector, a column and a detector. The principle of operation is simple. The pump pushes the eluent through the column at a certain flow rate. When injecting the sample, the eluent passes through the injector and transfers the sample in to the column. In the column, the sample components are separated components are detected at the detector. In modern LC instruments the operations are controlled by a computer. In most instruments it is possible to control the temperature of the eluent and column. In order to minimize peak broadening, the dead volume of the unit, especially in the injection system and in the detector must be kept small.

Figure no. 2 Structure of a HPLC unit with precolumn



Solvents

The selection of the eluent is dependent on the technique used. Generally, the solvent used in HPLC should be filtered before use, to remove suspended particles that could easily block the LC system. Dissolved gases should also be removed, either by out gassing with helium or nitrogen or by processing in an ultrasonic bath. The solvents used as the mobile phase are stores in a reservoir in glass or stainless steel bottles.

In HPLC, the separation can be achieved by either isocratic or gradient elution. In the isocratic method, the solvent composition is constant during the separation. Better separation in shorter analysis time is usually obtained by using gradient elution, in which the eluent composition is gradually changed during the analysis. Two or more eluents can be used in gradient elution and the gradient can be linear, stepwise, concave or convex. Typically, the elution strength of the solvent is increased in the gradient method. Thus, in RPLC the amount of organic solvent (e.g. acetonitrile) is increased while the amount of aqueous buffer is decreased.

Pumping systems

The requirements for a HPLC pump are high: The pump should be able to produce pressures up to 400 bar (40MPa) with a large range of flow rates (0.05 - 10 ml min^{-1}) and the flow should be free of pulsation. Also, the inner volume of the pump should be small enough to enable a quick change of the eluent composition. In addition, reproducibility and control of the flow with a relative error of less than 0.5% should be obtained. The most common pumps in HPLC systems are displacement and reciprocating pumps.

Stainless steel, Teflon or ceramics are used as materials. The high-pressure strain is obtained using sapphire valves. One differentiates between reciprocating and displacement pumps. *Displacement pumps* works like a syringe. A specific volume approximately 200 ml of the mobile phase is sucked in and then discharged free of pulsation in to the HPLC system. A crucial disadvantage however is the interruption of the delivery process in order to fill or rinse the piston. Displacement pumps are still used in micro-HPLC, since the composition of mobile phase during one analysis is small.

Reciprocating pumps are preponderant today. As a rule, they are operated as double-piston pumps, which work with a phase shift of 180˚ to suppress pulsation. They are also called oscillating (inverse) displacement pumps. To avoid direct contact with solvent with the pump valves, the pumps are also available as *piston diaphragm pumps.* Here, the piston movement is transferred to a diaphragm using hydraulics. The operation of the pumps is based on the movement of the piston, when the piston

moves backwards, it sucks eluent from the reservoir and on the forward movement and it pushes the eluent in to the column.

The advantage of the short piston pump are the small internal volumes of 40 to 400 µl, the high pressure outlet of up to 60 MPa, as well as the constant flow, which is independent of the back pressure of the column and the solvent viscosity.

Gradients can be produced on the low-pressure or high-pressure side. If one mixes the two or three compartment solvents of an eluent mixture on the suction side of a pump, one refers to a *low-pressure gradient.*

Two pumps are required to produce a *high-pressure gradient.* A single solvent constituent or in the case of ternary mixtures, two constituents are presented in a constant relationship. The third constituent is admixed-in on the pressure side of the pump after the gradient program.

High-pressure gradients are more precisely composed than low-pressure gradients. This can be ascribed to the fact that the volume contraction when the various solvents are mixed can become significant in the low-pressure variant.

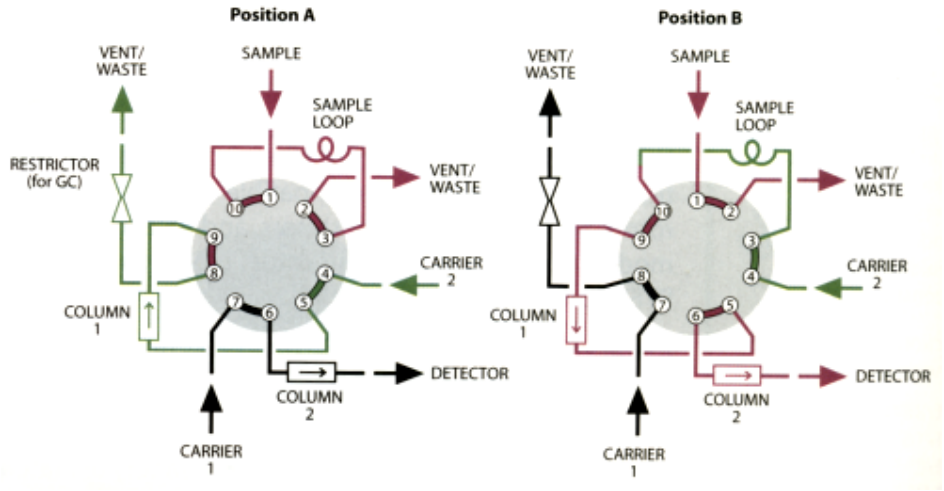
To avoid damaging the pump and contaminating the column particles, the sample solution should be filtered prior to injection e.g. by passing it through a 1 µm filter.

Injection System

The sample injection system must allow volumes in the range from 1 to 500 µl to be introduced. In micro-HPLC, the sample volumes are much lower (< 1 µl) and a different injection system is required than in conventional size HPLC. During the injection, the pressure should be kept constant in the system. The most commonly used injection system is presented in fig no. 3. It consists of a six-way valve, to which a sample loop has been attached. The sample is injected into the loop by means of micro liter syringe. While the loop is being filled, the eluent flows directly to the column. By switching the valve, the eluent flow is directed via the sample loop to the column. After injection, the valve is switched to its original position. The size of the

loop can be varied. In micro-HPLC systems, the sample loop is replaced with a groove in the valve, because it is difficult to produce sufficiently small loops.

Figure no. 3 Injection valve of HPLC



Automated sample injection systems are preferred for high-precision sample introduction. These are also based on sample loops and are operated by compressed-air switching. [1]

Columns

For most pharmaceutical analyses, separation is achieved by partition of compounds in the test solution between the mobile and stationary phases. A system consisting of polar stationary phases and non-polar mobile phases are described as normal phase, while the opposite arrangement, polar mobile phases and nonpolar stationary phases and is called reverse-phase chromatography. Partition chromatography is almost always used for hydrocarbon-soluble compounds of molecular weight less than 1000. The affinity of a compound for the stationary phase and thus its retention time on the column is controlled by making the mobile phase

more or less polar. Mobile phase polarity can be varied by the addition of a second and sometimes a third or even a fourth component.

Stationary phases for modern reverse-phase liquid chromatography typically consist of an organic phase chemically bound to silica or other materials. Particles are usually 3 to 10 μm in diameter, but sizes may range up to 50 μm or more for preparative columns. Small particles thinly coated with organic phase provide for low mass transfer resistance and hence rapid transfer of compounds between the stationary and mobile phases. Column polarity depends on the polarity of the bound functional groups, which range from relatively non-polar octadecyl silane to very polar nitrile groups. Liquid non-bound stationary phases must be largely immiscible in the mobile phase. Even so it is usually necessary to presaturate the mobile phase with stationary phase to prevent stripping of the stationary phase from the column. Polymeric stationary phases coated on the support are more durable.

Columns used for analytical separations usually have internal diameters of 2 to 5 mm; larger diameter columns are used for preparative chromatography. Columns may be heated to give more efficient separations, but only rarely are they used at temperatures above 60˚ because of potential stationary phase degradation or mobile phase volatility. Unless otherwise specified in the individual monograph, columns are used at ambient temperature.

In Size-exclusion chromatography the column is packed with a separation material that is capable of fractionation in the appropriate range of molecular sizes and through which the eluent is passed at a constant rate. One end of the column is usually fitted with a suitable device for applying the sample, such as a flow adaptor, a syringe through a septum or an injection valve and it may also be connected to a suitable pump for controlling the flow of the eluent. Alternatively, the sample may be applied directly to the drained bed surface or where the sample is denser than the eluent, it may be layered beneath the eluent. The packing material may be a soft support such as a swollen gel or a rigid support composed of a material such as glass, silica or a solvent-compatible, cross-linked organic polymer. Rigid supports usually require pressurized systems giving faster separations. The mobile phase is chosen according to sample type, separation medium and method of detection.

Ion-exchange chromatography is used to separate water-soluble, ionizable compounds of molecular weight less than 1500. The stationary phases are usually synthetic organic resins; cation-exchange resins contain negatively charged active sites and are used to separate basic substances such as amines, while anion-exchange resins have positively charged active sites for separation of compounds with negatively charged groups, such as phosphate, sulfonate or carboxylate groups. Water-soluble ionic or ionizable compounds are attracted to the resins and differences in affinity bring about the chromatographic separation. The pH of the mobile phase, temperature, ion type, ionic concentration and organic modifiers affect the equilibrium and these variables can be adjusted to obtain the desired degree of separation.[1]

Detectors

Ultraviolet/visible (UV/Vis) spectrophotometers, including diode array detectors are the most commonly employed detectors. Fluorescence spectrophotometers, differential refractometers, electrochemical detectors, mass spectrometers, light scattering detectors, radioactivity detectors or other special detectors may also be used. [10]

- ***Absorbance detectors***

  Many compendial HPLC methods require the use of spectrophotometric detectors. Such a detector consists of a flow-through cell mounted at the end of the column. A beam of UV radiation passes through the flow cell and into the detector. As compounds elute from the column, they pass through the cell and absorb the radiation, resulting in measurable energy level changes.

  Fixed, variable and multi-wavelength detectors are widely available.

  Fixed wavelength detectors - It is operate at a single wavelength typically 254 nm emitted by a low-pressure mercury lamp.

Variable wavelength detectors - It contains a continuous source, such as a deuterium or high-pressure xenon lamp and a monochromator or an interference filter to generate monochromatic radiation at a wavelength selected by the operator. The wavelength accuracy of a variable-wavelength detector equipped with a monochromator should be checked by the procedure recommended by its manufacturer; if the observed wavelengths differ by more than 3 nm from the correct values; recalibration of the instrument is indicated.

Modern variable wavelength detectors - This can be programmed to change wavelength while an analysis is in progress.

Multi-wavelength detectors - This measure the absorbance at two or more wavelengths simultaneously.

Diode array multi-wavelength detectors - In this continuous radiation is passed through the sample cell and then resolved into its constituent wavelengths, which are individually detected by the photodiode array. These detectors acquire absorbance data over the entire UV-visible range, thus providing the analyst with chromatograms at multiple, selectable wavelengths and spectra of the eluting peaks. Diode array detectors usually have lower signal-to-noise ratios than fixed or variable wavelength detectors and thus are less suitable for analysis of compounds present at low concentrations.

- ***RI detector (refractometers)***

Differential refractrometer detectors measure the difference between the refractive index of the mobile phase alone and that of the mobile phase containing chromatographed compounds as it emerges from the column. Refractive index detectors are used to detect non-UV absorbing compounds, but they are less sensitive than UV detectors. They are sensitive to small changes in solvent composition, flow rate and temperature, so that a reference column may be required to obtain a satisfactory baseline. [11]

- ***Fluorescence detectors***

Compared with UV detectors, up to about 1000-fold higher sensitivity can be achieved using fluorescence detectors. In fluorescence detectors the excitation source is most frequently a mercury vapor lamp. Xenon high-pressure lamps are also employed for more demanding tasks. In addition, the excitation and emission wavelengths can be selected by monochromators or a fluorescence spectrometer can be used as a detector. The intrinsic fluorescence of substances can often be exploited in analysis of drugs, of clinically relevant compounds or of natural substances. To detect non-fluorescent compounds the substances to be determined first have to be derivatized. [1]

- ***Electrochemical detectors***

Voltammetry, amperometry, coulometry and conductimetry can be exploited for electrochemical detection.

These detectors are selective, sensitive and reliable, but require conducting mobile phases free of dissolved oxygen and reducible metal ions. Pulseless pump must be used and care must be taken to ensure that the pH, ionic strength and temperature of the mobile phase remain constant. Working electrodes are prone to contamination by reaction products with consequent variable responses.

Electrochemical detectors with carbon-paste electrodes may be used advantageously to measure nanogram quantities of easily oxidized compounds, notably phenols and catechols. [11]

- ***Evaporative light scattering detector (ELSD)***

Detection is based on the scattering of a beam of light by particles of compound remaining after evaporation of the mobile phase. This detector is of growing importance; it is a universal and does not require a compound to have a chromophore for detection. Application includes the analysis of surfactants, lipids and sugars. Unlike the refractive index detector, which was formerly used for this analysis, it can be used with gradient elution and is robust enough

to function under a wide range of operating conditions. However, it cannot be used with in-volatile materials such as buffers in the mobile phase or to detect very volatile analytes. Typical applications include: analysis of chloride and sodium ions in pharmaceuticals, lipids used as components in formulations, sugars and sugar polymers. [4]

- ***Mass spectrometer***

The mass spectrometer is a very important HPLC detector because of its ability to generate structural and molecular weight information about the eluted solutes. The combination of HPLC and mass spectrometry allows for both separation and identification in the same step, an advantage none of the other detectors provide.

The major difficulty in using mass spectrometry is in designing the interface. The flow rate in HPLC is on the order of 1 ml/min, which are two or three orders of magnitude larger than the flow that can be taken by the conventional mass spectrometer vacuum systems. A second problem with using mass spectrometry is the difficulty of vaporizing non-volatile and thermally labile molecules without degrading them. [8]

Data Collection Devices

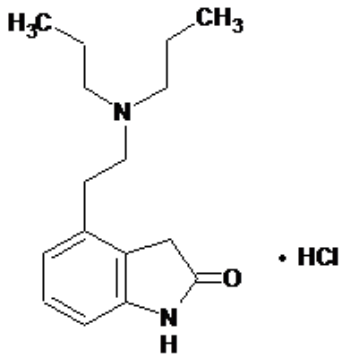
Modern data stations receive and store detector output and print out chromatograms complete with peak heights, peak areas, sample identification and method variables. They are also used to program the liquid chromatograph, controlling most variables and providing for long periods of unattended operation.

Data also may be collected on simple recorders for manual measurement or on stand-alone integrators, which range in complexity from those providing a printout of peak areas to those providing chromatograms with peak areas and peak heights calculated and data stored for possible subsequent reprocessing. [11]

2. DRUG PROFILE

2.1 NOMENCLATURE

Structure



CAS Registry number

91374-20-8

ATC Code

N04BC04

IUPAC Name

4-[2-(dipropylamino) ethyl]-1, 3-dihydro-2H-indol-2-one

Molecular Formula

$C_6H_{24}N_2O$. HCl

Molecular Weight

296.84 g/mol [12]

Percent Composition of Atoms

C – 64.74 %, H – 8.49 %, N – 9.44 %, O – 5.39 %, Cl – 11 % [13]

2.2 PHYSICAL AND CHEMICAL PROPERTIES

Description

White to pale greenish yellow crystalline powder

Distribution coefficient (Octanol/ Water)

Log P = 3.32 in Phosphate buffer at pH 7.4

Dissociation Constant (pKa)

9.5 for tertiary amine

11.6 for indole nitrogen

Solubility

Soluble in water and in dilute Hydrochloric acid

Slightly soluble in ethyl alcohol

Insoluble in methylene chloride

Melting Point

241 to 243 ˚C

Storage

Store at 20 to 25 ˚C, Protected from light.

2.3 PHARMACEUTICAL FORMS:

In India Ropinirole Hydrochloride is available in the range of 0.25 mg, 0.5 mg, 1 mg and 2mg as film coated tablets and 4mg as extended release tablets.

2.4 PHARMACOLOGY

Indication

- It is used in the management of Parkinson's disease, either alone or as an adjunct to levodopa.
- It is used in the treatment of Restless Leg Syndrome (RLS). [14]

Mechanism of Action

Ropinirole is a non-ergoline dopamine agonist with high relative in vitro specificity and full intrinsic activity at the D_2 and D_3 dopamine receptor subtypes, binding with higher affinity to D_3 than to D_3 or D_4 receptor subtypes.

Dosage

- ***As monotherapy in Parkinson's disease:***

  Adult: Initially, 250 μg tid may increase by 750 μg at weekly intervals for the first 4 week. Subsequent increments can be made in steps of 1.5 mg at weekly intervals up to 9 mg/day, then in steps of 3 mg at weekly intervals. Usual dose ranges from 3-9 mg daily. Max: 24 mg/day. Higher dose may be necessary if used in conjunction with levodopa.

- ***Restless leg syndrome:***

  Adult: Initially, 250 μg daily for 2 days, taken 1-3 hr before bedtime. May increase to 500 μg daily for the next few days, subsequent increments may be made in steps of 500 μg at weekly intervals until 3 mg daily is reached. Maximum dose: 4 mg daily. [15]

Contraindication

Contraindicated for patients known to have hypersensitivity reaction (including urticaria, angioedema, rash, pruritus) [16]

Adverse Drug Reactions

Nausea, vomiting, somnolence, insomnia, dyspepsia, dizziness, hallucinations, tremors, abdominal pain, depression, headache, edema of the legs, ataxia, anxiety and symptomatic hypotension [17] [18]

Drug - Drug Interactions

Ciprofloxacin : Inhibition of ropinirole metabolism

Antipsychotics : Antagonise the effect of ropinirole

Memantine : Enhancing the effect of ropinirole

Methyldopa : Antagonise the effect of ropinirole

Metoclopramide : Antagonise the effect of ropinirole

Oestrogens : Increase in the plasma concentration of ropinirole [12]

Pharmacokinetics

Absorption : Rapidly absorbed from the GI tract

Bioavailability : About 50%

Distribution : Widely distributed

Plasma protein binding : 10-40%

Metabolism : Extensively metabolized in the liver by CYP1A2

Excretion : Excreted in the urine as inactive metabolites; less than 10% of the oral dose is excreted unchanged

Elimination half-life : About 6 hours [19] [20] [21]

3. LITERATURE REVIEW

3.1 Spectroscopic Methods

- **M.V.Kumudhavalli et al. [22] [2011]** have developed and reported a validated spectrophotometric method for the determination of Ropinirole in pharmaceutical formulation. Distilled water was used as a solvent throughout the study. Quantitative determination of Ropinirole in pharmaceutical formulation was carried out by UV spectrophotometric method using λ max at 249.0 nm. The method showed high specificity in the presence of formulation excipients and good linearity in the concentration range of 10-30 µg/ml. Percentage recovery values at 249.0 nm were 96 to 101.30% .The intra and interday precision data demonstrated that method was precised. The method was validated in terms of accuracy, precision and specificity. The method could be routinely adopted for quality control of these drugs in tablet.

- **Sudarshan Purohit et al. [23] [2010]** have reported a simple, sensitive and selective UV spectroscopy method for the estimation of ropinirole HCl in pharmaceutical formulation. Estimation of drug was performed in 0.1 N HCl at 230 nm. The validation studies were carried out with reference to ICH requirements. The developed method was found to be specific, linear, precise (including both intra- and inter-day), accurate and robust. This proposed method might represent a valuable aid in the routine quality estimation of ropinirole HCl.

- **Vishnu P. Choudhari et al. [24] [2010]** have described two simple, precise and economical UV spectrophotometric methods for the estimation of ropinirole in pharmaceutical dosage form. In Method (A) area under curve (AUC) tool was applied and in which area under curve was integrated in the wavelength range of 234.36 – 241 nm. Method (B) involved first order derivative spectrum of drug solution and measurement of derivative amplitude at 262.58 nm. Calibration curves were plotted for both methods by using instrumental response at selected wavelength and concentrations of analyte in the solution. Linearity for the detector

response was observed in the concentration range of 4-20 µg/ml for both the methods. Two tablet formulations were analyzed and percentage assay determined was 99.79% – 100.68%. Accuracy and precision studies were carried out and results were satisfactory. The proposed methods were validated as per ICH analytical method development guidelines. The results of the analysis were validated statistically. Limit of detection and limit of quantitation were determined for both methods.

- **Shete Yogita et al. [25] [2009]** have developed and reported a simple, sensitive, rapid, accurate and precise spectrophotometric method for estimation of ropinirole hydrochloride in bulk and tablet dosage forms. Ropinirole hydrochloride showed maximum absorbance at 250 nm with molar absorptivity of 8.703×10 3 l/mol.cm. Beer's law was obeyed in the concentration range of 5-35 µg/ml. Results of analysis were validated statistically and by recovery studies.

- **Aydogmus Zeynep [26] [2008]** has reported three sensitive, selective, accurate spectrophotometric and spectrofluorimetric methods for the determination of ropinirole hydrochloride in tablets. The first method was based on measuring the absorbance of drug solution in methanol at 250 nm. The Beer's law was obeyed in the concentration range 2.5–24 µg ml−1. The second method was based on the charge transfer reaction of drug, as n-electron donor with 7,7,8,8-tetracyanoquinodimethane (TCNQ), as π-acceptor in acetonitrile to give radical anions that were measured at 842 nm. The Beer's law was obeyed in the concentration range 0.6–8 µg ml−1. The third method was based on derivatization reaction with 4-chloro-7-nitrobenzofurazan (NBD-Cl) in borate buffer of pH 8.5 followed by measuring the fluorescence intensity at 525 nm with excitation at 464 nm in chloroform. Calibration curve was constructed in the concentration range 0.01-1.3 µg ml^{-1}. The derivatization reaction product of drug with NBD-Cl was characterized by IR, 1H NMR and mass spectroscopy. The developed methods were validated by parameters such as the molar absorptivity, limit of detection (LOD, µg ml^{-1}) and limit of quantitation (LOQ, µg ml^{-1}), precision, accuracy, recovery, and Sandell's sensitivity. Selectivity was validated by subjecting stock

solution of ropinirole to acidic, basic, oxidative and thermal degradation. No interference was observed from common excipients present in formulations. The proposed methods were successfully applied for determination of drug in tablets. The results of these proposed methods were compared with each other statistically.

- **Armagan Onal et al. [27] [2007]** have developed simple and reproducible spectrophotometric methods for the determination of dopaminergic drugs used for Parkinson's disease, cabergoline (CAB) and ropinirole hydrochloride (ROP), in pharmaceutical preparations. The methods were based on the reactions between the studied drug substances and ion-pair agents [methyl orange (MO), bromocresol green (BCG) and bromophenol blue (BPB)] and yellow colored ion-pair complexes were produced in acidic buffers; Then the ion-pair complexes were extracted in dichloromethane and which were spectrophotometrically determined at the appropriate wavelength of ion-pair complexes. Beer's law was obeyed within the concentration range from 1.0 to 35 μg ml−1. The developed methods were applied successfully for the determination of these drugs in tablets.

- **J. V. Susheel et al. [28] [2007]** have described a Ultra Violet Spectroscopy for the determination of ropinirole hydrochloride in tablet dosage forms. Detection wavelength was found to be 250 nm using ethanol as a solvent. For this method the linearity was found to be in the range of 5-30 μg/ml. The developed method could be applied for routine analysis of ropinirole hydrochloride from tablet dosage forms.

- **Mahaki Hanieh et al. [29] [2011]** have reported the interaction between ropinirole hydrochloride and Human serum albumin as binary system by Three-dimensional Fluorescence Spectroscopy. The emission wavelength was recorded between 300 and 600 nm, the initial excitation wavelength was set to 200 nm with increment of 10 nm. Peak (a) was the Rayleigh scattering peak (=) and peak (b) was the second-ordered scattering peak (= 2). The fluorescence intensity of peak (a) and peak (b) was increased with the addition of Ropinirole hydrochloride. The possible reason

was that a RP–HSA complex was formed after the addition of ropinirole hydrochloride followed by increasing the diameter of the macromolecule which in turn resulted in an enhanced scattering effect. Peak 1 mainly revealed the spectral behavior of Tryptophan and Tyrosine residues. The reason was that when HSA was excited at 280 nm, it mainly revealed the intrinsic fluorescence of Tryptophan and Tyrosine residues. Beside peak 1, there was another fluorescence peak 2 (= 230.0 nm, = 340.0 nm) that mainly reflected the fluorescence spectral behavior of the polypeptide backbone structure of HSA. The fluorescence intensity of peak 2 decreased after the addition of ropinirole hydrochloride, which showed that the peptide strands structure of HSA had been changed.

- **Hanieh Mahaki** [30] **[2011]** has reported a binding analysis of Ropinirole Hydrochloride and Aspirin to Human Serum Albumin by Synchronous Fluorescence. Fluorescence measurements were carried out on a F-2500 (Hitachi, Japan) with a 150W Xenon lamp spectrofluorimeter. Synchronous fluorescence gave information about the molecular environment in a vicinity of the chromophore molecule. The D-value (Δλ) between excitation and emission wavelengths was stabilized at 15 or 60 nm; the synchronous fluorescence gave the characteristic information of tyrosine or tryptophan residues. Fluorescence intensity decreased regularly with the addition of Ropinirole Hydrochloride and Aspirin. The synchronous fluorescence spectra of Human Serum Albumin with various amounts of Ropinirole Hydrochloride and Aspirin were recorded at Δλ=60 nm. The tryptophan fluorescence emission of Aspirin was decreased regularly, but no significant change in wavelength was observed. At the same time, the emission wavelength of the tryptophan residues was slight blue-shifted in Ropinirole hydrochloride.

3.2 Chromatographic Techniques

- **Sundaramurthy Poongothai et al. [31] [2011]** have developed a dissolution test for *in vitro* evaluation of tablet dosage forms containing 5 mg of ropinirole by reverse phase high performance liquid chromatography. A discriminatory dissolution method was established using apparatus basket at a stirring rate of 50 rpm with 500 ml of pH 4.0 deaerated citrate buffer as dissolution medium and detection was carried out at 250 nm. The retention time of ropinirole hydrochloride was found to be 3.84 minutes. The proposed method was validated to meet requirements for a global regulatory filing which included linearity, specificity, precision, accuracy, ruggedness and robustness and to evaluate the formulation during an accelerated stability study. The method could be applied for the quality-control analysis of ropinirole tablets. Moreover, quantitative analysis was also performed, to compare the applicability of the RP-LC and the LC-MS/MS methods.

- **Ch. Krishnaiah et al. [32] [2010]** have reported a novel stability-indicating gradient reverse phase ultra performance liquid chromatographic (RPUPLC) method for the determination of purity of ropinirole in presence of its impurities and forced degradation products. The method was developed using Waters Aquity BEH 100 mm, 2.1 mm, 1.7 µm C-8 column with mobile phase containing a gradient mixture of solvent A and B. The eluted compounds were monitored at 250 nm. The run time was within 4.5 min in which ropinirole and its four impurities were well separated. Ropinirole was subjected to the stress conditions of oxidative, acid, base, hydrolytic, thermal and photolytic degradation. Ropinirole was found to degrade significantly in oxidative and base stress conditions and stable in acid, water, hydrolytic and photolytic degradation conditions. The degradation products were well resolved from main peak and its impurities. Thus it proved the stability indicating power of the method. The developed method was validated as per International Conference on Harmonization (ICH) guidelines with respect to specificity, linearity, limit of detection, limit of quantification, accuracy, precision and

robustness. This method was also suitable for the assay determination of ropinirole in pharmaceutical dosage forms and dissolution studies.

- **N.Sreekanth et al. [33] [2009]** have described a simple and accurate RP-HPLC method has been developed for the estimation of ropinirole hydrochloride in bulk and pharmaceutical dosage forms using C_{18} column 250 x 4.6 mm i.d, 5µm particle size in isocratic mode, with mobile phase comprising of buffer (pH 6.0) and Acetonitrile in the ratio of 50:50 v/v. The flow rate was 0.5ml/min and detection was carried out by UV detector at 245nm. The retention time for Ropinirole Hydrochloride was found to be 4.867 min. The proposed method has permitted the quantification of ropinirole hydrochloride over linearity in the range of 5-50µg/ml and its percentage recovery was found to be 99.3-100.4%. The intraday and inter day precision were found 0.27% and 0.26% respectively.

- **Saral et al. [34] [2009]** have reported a validated RP-HPLC method for the estimation of Ropinirole hydrochloride in tablet dosage form and its IVIVC studies. The chromatographic conditions were,

  Column : Octadecyl carbon chain bonded silica column
  Mobile Phase : Phosphate buffer (pH 6.5): Acetonitrile (70:30)
  Flow rate : 1 ml min -1
  Detection : UV detection at 250 nm

  Linearity was found to be over a range of 25 to 150 % of actual concentration (r = 0.9999), with limit of detection and quantification of 0.062 and 0.186 µg ml^{-1}, respectively. The analytical method passed both robustness and ruggedness tests. In both cases, relative standard deviation was well satisfactory. The method could be used for quality control assay of ropinirole hydrochloride. The results obtained from the dissolution in different media showed that the release was almost similar in all the media but pH 4.0 citrate buffer was considered to be the discriminating media considering that the medium showed variation as a result of change in formulation of the drug. The

drug was freely and rapidly soluble in water, so the sink condition was not mandatory. The volume of dissolution media was selected as 500ml based on poor absorbance as that of 900ml. After optimizing, the complete method validation was made with various parameters. The dissolution data i.e. percentage drug released at 15 minutes which have a close linear relationship with correlation coefficient as 0.9968 and slope around unity with the biological property i.e. C_{max}.

- **B. Jancic-Stojanovic et al. [35] [2009]** have reported a chemometrical evaluation of ropinirole and its impurity's (4-[2-(dipropylamino) ethyl]-1H-indol-2, 3-Dione) chromatographic behavior in systematic and the most efficient way. Face-centered central composite design (CCD) with 23 full factorial designs, ±1 star design and four replication in central point was applied for a response surface study, in order to examine in depth the effects of the most important factors. Factors—independent variables (acetonitrile content, pH of the mobile phase and concentration of sodium heptane sulfonate in water phase) were extracted from the preliminary study and as dependent variables five responses (retention factor of ropinirole, retention factor of its impurity, resolution, symmetry of ropinirole peak and symmetry of impurity peak) were selected. For the improvement of method development and optimization step, Derringer's desirability function was applied simultaneously to optimize the five chosen responses. The procedure allowed deduction of optimal conditions and the predicted optimum was acetonitrile-5 mM of sodium heptane sulfonate (21.6:78.4, v/v), pH of the mobile phase adjusted at 2.0 with ortho phosphoric acid. By calculating global desirability's determination coefficients (), as well as by the visual inspection of 3D graphs for global desirability, robustness of the proposed method was also estimated.

- **A. Azeem et al. [36] [2008]** have disentangled an accurate, sensitive, precise, rapid, and isocratic reversed phase High performance liquid chromatography (RP-HPLC) method for analysis of ropinirole in the bulk drug and in pharmaceutical preparations. The best separation was achieved on a 250 mm × 4.6 mm i.d, 5-µm particle, C_{18} reversed-phase column with methanol: 0.05 M

ammonium acetate buffer (pH 7) 80:20 (v/v) as mobile phase, at a flow rate of 1 ml min^{-1}. UV detection was performed at 250 nm. The method was linear over the concentration range 0.2–100 μg ml^{-1} (r = 0.9998), with limits of detection and quantitation of 0.061 and 0.184 μg ml^{-1}, respectively. The drug was subjected to oxidation, hydrolysis, photolysis, and heat as stress conditions. Degradation products resulted from the stress did not interfere with detection and assay of ropinirole and thus the method could be regarded as stability-indicating. The method could be used for quality-control assay of ropinirole.

- **B. Sahasrabuddhey et al. [37] [2007]** have isolated three impurities in ropinirole hydrochloride drug substance at levels 0.06–0.15% were detected by using reverse-phase high performance liquid chromatography (HPLC). These impurities were analyzed using reverse-phase HPLC. Based on the spectral data (IR, NMR and MS), structures of these impurities were characterized as 4-[2-(propylamino) ethyl]-1,3-dihydro-2H-indol-2-one hydrochloride (impurity-A), 5-[2-(dipropylamino) ethyl]-1,4-dihydro-3H-benzoxazin-3-one hydrochloride (impurity-B) and 4-[2-(dipropylamino) ethyl]-1H-indol-2,3-dione hydrochloride (impurity-C). Synthesis of these impurities was discussed.

  The present study was illustrated the isolation of three process related unknown impurities of ropinirole hydrochloride by preparative HPLC which were further characterized using various spectroscopic techniques.

- **Armagan Onal [38] [2006]** have explicated a reversed-phase high-performance liquid chromatographic (HPLC) method with UV detection for the determination of ropinirole (ROP) in tablets. The assay utilized UV detection at 250 nm and a Luna CN column (250 × 4.6 mm I.D, 5 μm). The mobile phases were comprised of acetonitrile: 10 mM nitric acid (pH 3.0) (75:25, v/v). Validation experiments were performed to demonstrate linearity, accuracy, precision, limit of quantitation (LOQ), limit of detection (LOD), and robustness. The method was linear over the concentration range of 0.5–10.0

μg mL−1. The method showed good recoveries (99.75–100.20%) and the relative standard deviations of intra and inter-day assays were 0.38–1.69 and 0.45–1.95%, respectively. The method could be used for quality control assay of ropinirole.

- **Jeffery Hackett [39] [2006]** has evaluated solid-phase sorbents for the analysis of ropinirole in whole blood. In this method, drug free blood was spiked with ropinirole (0 to 10 ng) and an internal standard (quinidine). The samples were buffered with distilled water and centrifuged. The supernatant liquid was applied to previously conditioned end capped C_6, C_{18} and C_8/SCX solid phase extraction columns. The columns were washed, dried, and eluted with various solvents systems. The eluants were collected and evaporated. The residue was dissolved in 100 μl of aqueous 0.1% trifluoroacetic acid and analyzed by liquid chromatography using a C_{18} (4.6 × 150 mm, 5-μm particle size) column and monitored at 250 nm, using diode-array detection. A mobile phase consisting of methanol/0.1% TFA in distilled water (22:78 v/v) was employed. The data was collected and appraised. It was found that 3-ml 200-mg CEC06 C_6 (Hexyl end capped) solid-phase columns that had been washed with 3 × 3 ml water and 3 × 3 ml acetonitrile and eluted with a solvent system consisting of 95:5 v/v acetonitrile/ammonia performed best. The linear range for this analysis was found to be from 0 to 10 ng/ml. The limit of detection was determined to be 1 ng/ml with a limit of quantification of 2.5 ng/ml.

- **George Lunn [40] [2005]** has reported a liquid chromatographic determination of ropinirole hydrochloride in rat, dog and human plasma.

Column	: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase	: ACN: 70 mM pH 3.8 ammonium formate buffer (25:75) containing 0.3% EDTA and 0.005% SOS
Flow rate	: 1ml/min
Injection volume	: 10–100 μl
Detector	: UV 250 nm

Retention time : 9.4 min
Internal standard : 4-(2-di-*N*, *N*-propylaminoethyl)-7-methoxy-2-(3H)-Indoline HCl (11.5 min)
Limit of detection : 5 ng/ml
Limit of quantitation : 10 ng/ml

- **Pavel Coufal et al. [41] [1999]** have reported a Capillary liquid chromatography (CLC) for the separation and quantification of ropinirole and its five related impurities, potentially formed during its synthesis. A simultaneous optimization of three mobile phase parameters, i.e., pH, buffer concentration and acetonitrile content was performed employing an experimental design approach which proved a powerful tool in method development. The retention factors of the investigated substances in different mobile phases were determined. Baseline resolution of the six substances on a C_{18} reversed stationary phase was attained using a mobile phase with an optimized composition [acetonitrile-8.7 mM 2-(N-morpholino) ethanesulfonic acid adjusted to pH 6.0 (55:45, v/v)]. It was shown that CLC, operated in the isocratic mode under the mobile phase flow-rate of 4µl/min, could determine the level of these impurities, down to a level of 0.06% of the main component within 25 min.

- **SB Bari et al. [42] [2011]** have developed and ratified a TLC/densitometry of ropinirole hydrochloride as a bulk drug. The separation was achieved on TLC aluminium plates precoated with silica gel 60F-254 as the stationary phase using chloroform: acetone: triethylamine (3.5:1.5:0.2 v/v) as mobile phase and densitometry analysis at 250 nm. The system showed compact spot for ropinirole hydrochloride (Rf = 0.52 ± 0.02). The drug followed linearity in the concentration range 300 - 1800 ng per band (r2 = 0.9983 ± 0.0008). Drug was subjected to hydrolysis, oxidation and thermal degradation which indicate the drug is susceptible to hydrolysis, oxidation and heat and degraded product did not interfere with detection and assay of ropinirole hydrochloride. Statistical analysis proved that the method was repeatable, selective and accurate for the estimation of ropinirole hydrochloride.

- **Gulam Mustafa et al. [43] [2011]** have developed and validated a stability-indicating high-performance thin-layer chromatographic (HPTLC) method for analysis of ropinirole HCl as per the ICH guidelines. The method employed the mobile phase, toluene-ethyl acetate-6M ammonia solution (5:6:0.5, v/v/v). Densitometric analysis of ropinirole HCl was carried out in the absorbance mode at 250 and 254 nm. Compact spots for ropinirole HCl were found at Rf value of 0.58 ± 0.02. The linear regression analysis data for the calibration plots showed R2 = 0.9989 ± 0.0053 with concentration range of 100– 3000 ng spot-1. The method was validated for precision, accuracy, ruggedness, robustness, specificity, recovery, limit of detection (LOD) and limit of quantitation (LOQ). The LOD and LOQ were 12.95 and 39.25 ng spot-1 respectively. Drug was subjected to acidic, alkaline, oxidative, dry heat, wet heat and photo degradation stress. All the peaks of degradation products were well resolved from the standard drug peak with significantly difference of retention factor. The acidic and alkaline stress degradation kinetics of ropinirole, was found to be in first order, showing high stability (t1/2,146.37 hr-1; t0.9, 39.11 hr-1) in acidic medium and low stability (t1/2,97.67 hr-1; t0.9, 14.87 hr-1) in alkaline environment.

- **T. K. Ravi et al. [28] [2007]** have developed a High Performance Liquid chromatography (HPTLC) for the determination of ropinirole hydrochloride in tablet. Solvent system used here was Methanol: Acetonitrile (8: 2 v/v) Detection wave length was found to be 254 nm. The linearity was found to be between 40 to 120 μg/ml. Since aripiprazole peak was well resolved from ropinirole hydrochloride peak and had good peak shape, aripiprazole was selected as an internal standard. This method could be applied for routine analysis of ropinirole hydrochloride in tablet dosage forms.

3.3 Electrophoretic techniques:

- **Pavel Coufal et al. [44] [1998]** have reported a Capillary Zone Electrophoresis (CZE) method for the determination of the dissociation constants of ropinirole and five structurally related impurities, potentially formed during its synthesis and for separation and quantification of these substances. The dissociation constants obtained from the CZE measurements were confirmed by UV spectrophotometry for some of the test compounds, obtaining a good agreement between the values. Careful optimization of the running buffer composition permitted base-line resolution of the six compounds in a borate buffer containing acetonitrile and magnesium sulfate (a 100 mM borate buffer containing 30 mM $MgSO_4$ and 20 vol. % of 4 acetonitrile). It was shown that CZE could determine the level of these impurities, down to a level of 0.05% of the main component within 15 min.

3.4 Hyphenated Techniques:

- **D. Vijaya Bharathi et al. [45] [2009]** have explicated a highly sensitive, rapid assay method has been developed and validated for the estimation of ropinirole (RPR) in human plasma with liquid chromatography coupled to tandem mass spectrometry with electro spray ionization in the positive-ion mode. A solid-phase process was used to extract RPR and citalopram (internal standard, IS) from human plasma. Chromatographic separation was operated with 0.2% ammonia solution: acetonitrile (20:80, v/v) at a flow rate of 0.50 ml/min on a Hypurity C_{18} column with a total run time of 3.2 min. The MS/MS ion transitions monitored were 261.2 → 114.2 for RPR and 325.1 → 209.0 for IS. Method validation and clinical sample analysis were performed as per FDA guidelines and the results met the acceptance criteria. The lower limit of quantitation achieved was 3.45 pg/ml and the linearity was observed from 3.45 to 1200 pg/ml. The intra-day and inter-day precisions were in the range of 4.71-7.98 and 6.56-8.31%, respectively. This novel method had been applied to a pharmacokinetic study of RPR in humans.

- **Erin E. Chambers et al. [46] [2008]** have evolved a rapid and sensitive SPE-UPLC/MS/MS method for the determination of ropinirole hydrochloride in human plasma. Citalopram is used as an internal standard.

  LC Conditions:

Column	: C_{18}
Mobile Phase	: 10mM NH_4COOH (pH 9): CH_3OH
Flow Rate	: 0.5ml/min
Injection volume	: 8.0 µl
Column Temperature	: 45˚C

  MS Conditions:

Ion Source	: Electro spray positive (ESI^+)
Desolvation Temperature	: 350˚ C
Cone gas flow	: 50 L/Hr
Desolvation gas flow	: 750 L/Hr
Collision cell pressure	: $2.6 \times 10^{(-3)}$ mbar

  This method achieved a S/N of over 100: 1 at the required LLOQ of 0.005ng/ml. the method meets the FDA requirements for linearity and excellent recovery for both analytes. This method enables researchers to obtain higher quality data faster in order to make critical project decisions.

- **Ai-Dong Wen et al. [47] [2007]** have examined the effect of Madopar on the pharmacokinetics of ropinirole in healthy Chinese volunteers by using liquid chromatography tandem mass spectrometry (HPLC/MS/MS). A single dose of 1mg ropinirole was given orally after administration of the placebo or Madopar (containing 200 mg levodopa and 50 mg benserazide) to six healthy males and six healthy females in a cross-over randomized study with a

minimum washout period of 8 days. Pharmacokinetic parameters were calculated for both treatments. Co administration of ropinirole and Madopar did not result in a notable change in rate or extent of availability of ropinirole, as shown by the ratios (90% confidence intervals) of 1.045 (0.900, 1.222) for C_{max} (maximum plasma concentration) and 1.167 (1.086, 1.262) for AUC_{0-inf} (the area under the concentration–time curve). Likewise, no significant difference in any of the other pharmacokinetic parameters [T_{max} (the time needed to reach the C_{max}), MRT (mean residence time), volume of distribution (V/F), and clearance (CL/F)] was observed between the treatment groups. No clinically relevant adverse effects were detected under either conditions and there are no pharmacokinetic grounds for adjusting the dose of ropinirole when given in combination with Madopar in Chinese patients.

- **William Edgemond et al. [48] [2007]** have elaborated a LC-MS-MS method for the quantitation of ropinirole in human plasma. The method was validated with a quantitative range of 10.0 to 1000 pg/ml. EDTA human plasma (0.5 ml) was fortified with internal standard, ropinirole-D_3 prior to extraction. After addition of sodium carbonate solution, the samples were extracted with ethyl acetate/cyclohexane, 9:1. After evaporating the solvent, the samples were reconstituted in mobile phase (A). A Hypersil GOLD PFP (3& [mu], 50x4.6 mm) column was used, yielding a retention time of 1.5 minutes. A step gradient method was used to clear late eluters (mobile phase A consisted of 10 mM ammonium acetate in 1:1, methanol: water and mobile phase B consisted of 100% methanol). Detection was carried out on a SCIEX API-5000 LC-MS-MS in positive Ion Spray MRM mode. The transitions monitored were m/z 261 & [rarr] 114 for ropinirole and m/z 264 & [rarr] 117 for ropinirole-D3. Three validation runs were performed on separate days. Precision (%CV) and accuracy (%bias) across all levels of the range were within & [plusmn] 8.0%. The precision and accuracy at the LLOQ was within & [plusmn] 9.0%. Extraction recovery ranged from 86% to 93%. No chromatographic interferences or matrix effects from six different lots of plasma were observed indicating the specificity of the method. Stability of ropinirole in plasma was established for 24 hours at room temperature, for 5 cycles of freezing and

thawing, and for 141 hours in the final extract. Long term stability of ropinirole in plasma was shown to be 80 days at –20 & [deg] C. The step gradient improved the robustness of the method. This method was successfully validated. The method proved rugged and sensitive in the determination of the concentrations of ropinirole in over 3400 samples generated from clinical trials.

- **Jignesh Bhatt et al. [49] [2006]** have reported a rapid and robust liquid chromatography-mass spectrometry (LC-MS/MS) method for non-ergoline dopamine D (2)-receptor agonist, ropinirole in human plasma using Es-citalopram oxalate as an internal standard. The method involves solid phase extraction from plasma, reversed-phase simple isocratic chromatographic conditions and mass spectrometric detection that enables a detection limit at picogram levels. The proposed method was validated with linear range of 20-1,200 pg/ml. The extraction recoveries for ropinirole and internal standard were 90.45 and 65.42%, respectively. The R.S.D. % of intra-day and inter-day assay was lower than 15%. For its sensitivity and reliability, the proposed method was particularly suitable for pharmacokinetic studies.

4. AIM AND PLAN OF THE WORK

4.1 Aim of the Work

The aim of the present study was to develop newer analytical methods for the estimation of ropinirole hydrochloride in bulk drug and its formulations. Ropinirole hydrochloride is an indole derivative and has di-alkylated tertiary amine in its side chain.

Literature survey reveals that only few analytical methods have been developed and reported for the estimation of ropinirole hydrochloride; they were UV, UPLC, HPLC, HPTLC, TLC, CZE, LC-MS and SPE-UPLC-MS.

As discussed earlier in drug profile, ropinirole hydrochloride is highly soluble in aqueous solvents. So the present study aims to develop newer and sensitive methods for the analysis of ropinirole hydrochloride using simple and economic aqueous solvents.

4.2 Plan of the Work

Development of newer analytical methods as follows:

- UV Spectrophotometric determination of ropinirole hydrochloride using 0.1 M acetic acid as solvent.
- Extractive spectrophotometric estimation of ropinirole hydrochloride using 0.2 % picric acid in water used as a reagent.
- RP - HPLC method for the estimation of ropinirole hydrochloride using ODS column as stationary phase and potassium dihydrogen phosphate buffer in 10 % ortho phosphoric acid (pH 3.3): acetonitrile (70: 30) as mobile phase.

5. METHODOLOGY

5.1 UV Spectrophotometric Determination of Ropinirole Hydrochloride using 0.1 M Acetic acid

Apparatus/Instruments Used

UV- Visible double beam Spectrophotometer (Perkin Elmer EZ 301)

Analytical electronic weighing balance (Shimadzu)

Vortex mixer

Solvents Used

Acetic acid - AR Grade

Water - Distilled Water

Reference Standard

Ropinirole hydrochloride was obtained as a gift sample from East West Pharma Haridwar - 247 667, India.

Tablet Formulations

Ropark film coated tablets – 2 mg (Sun Pharmaceuticals Industries Ltd)

Ropin film coated tablets – 2 mg (East West Pharma)

Preparation of 0.1 M Acetic acid in Water

It was prepared by diluting 5.7 ml of concentrated acetic acid in 1000 ml of distilled water.

Preparation of standard stock solution

Standard stock solution - I

Standard stock solution - I was prepared by dissolving 25 mg of ropinirole hydrochloride working standard in 25 ml of 0.1 M acetic acid to get a final concentration of 1 mg/ml.

Standard stock solution - II

2 ml of standard stock solution I was taken and further diluted to 25ml with same solvent to get the final concentration of 80 μg/ml.

Selection of Wavelength

2.5 ml of standard stock solution - II was pipetted out and diluted to 10ml with 0.1 M acetic acid. Then this solution was scanned in the UV region of 200 - 400 nm to get absorption maximum (λ_{max}). The drug was found to have a maximum absorbance at 250 nm in 0.1 M acetic acid and hence this wavelength was selected for further studies. The absorbance data were presented in table no.2 and absorption spectrum was shown in figure no.4.

Table no: 2 Data for the absorption spectrum of ropinirole hydrochloride using 0.1 M acetic acid

S.No	Wavelength	Absorbance
1	200	3.000
2	210	3.000
3	220	0.711
4	230	0.328
5	240	0.502
6	250*	0.625
7	260	0.425
8	270	0.140
9	280	0.101
10	290	0.056
11	300	0.008
12	310	0.004
13	320	0.004
14	330	0.003
15	340	0.003
16	350	0.002
17	360	0.003
18	370	0.003
19	380	0.003
20	390	0.004
21	400	0.003

Figure no: 4 Absorption spectrum of ropinirole hydrochloride using 0.1 M acetic acid

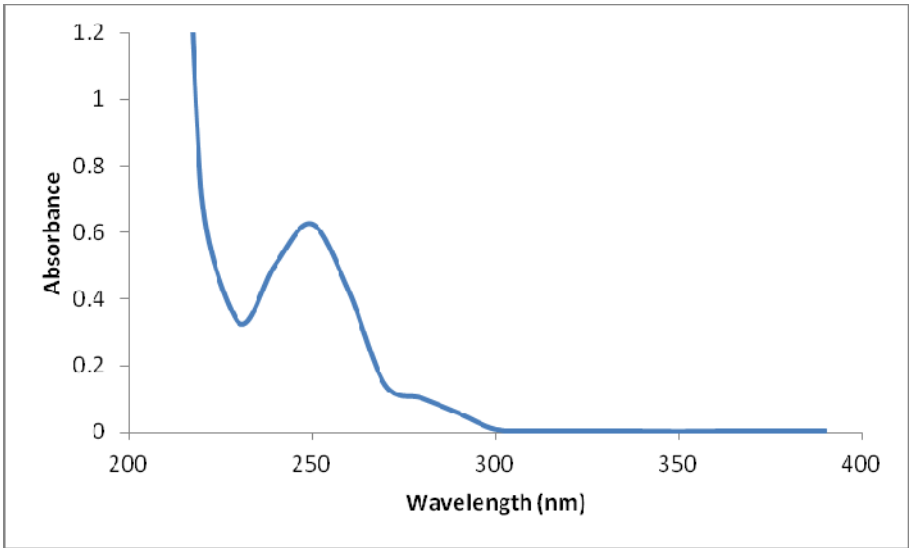


Table no: 3 System Parameters

Wavelength Scan	
Data Mode	Absorbance
Start WL (nm)	400.00
Stop WL (nm)	200.00
Scan Speed (nm/min)	100
Slit Width (nm)	1.5
Path Length (nm)	10.0

Beer's law plot

This law states that, when a beam of monochromatic radiation is passed through a solution of an absorbing substance, the rate of increase of intensity of radiation of thickness of the absorbing solution proportional to the intensity of incident radiation as well as the concentration of the solution.

Mathematically, this law was stated as

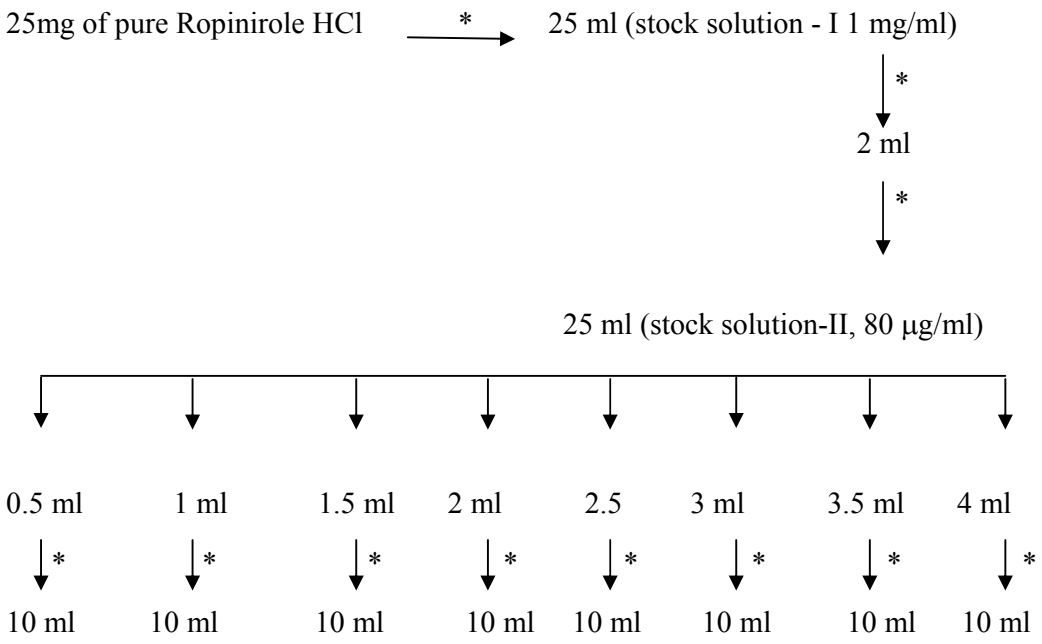
$$- dI/dx = k' Ic$$

Construction of calibration curve

Aliquots of working standard stock solution - II ranging from (0.5ml to 4ml) were transferred into a series of 10ml volumetric flasks. Then the volume was made up with 0.1 M acetic acid. The absorbance of resulting solution of different concentration was measured at 250nm against the solvent blank. Calibration curve was constructed by plotting absorbance vs. concentration.

Dilution chart

Dilution chart of calibration curve

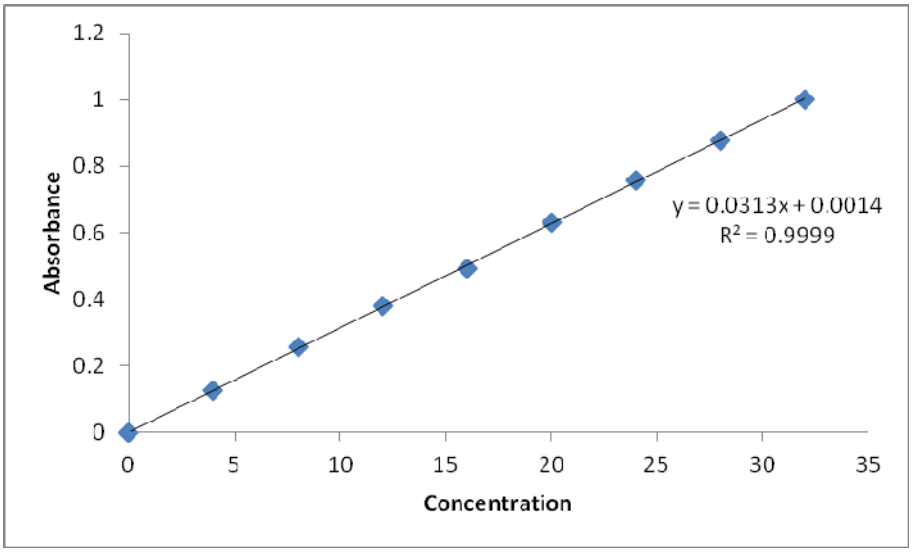


* 0.1 M Acetic acid

Table no: 4 Data for calibration curve of ropinirole hydrochloride using 0.1 M acetic acid

Concentration (µg/ml)	Absorbance
4	0.126
8	0.255
12	0.380
16	0.494
20	0.631
24	0.758
28	0.877
32	1.002

Figure no: 5 Calibration curve of ropinirole hydrochloride using 0.1 M acetic acid

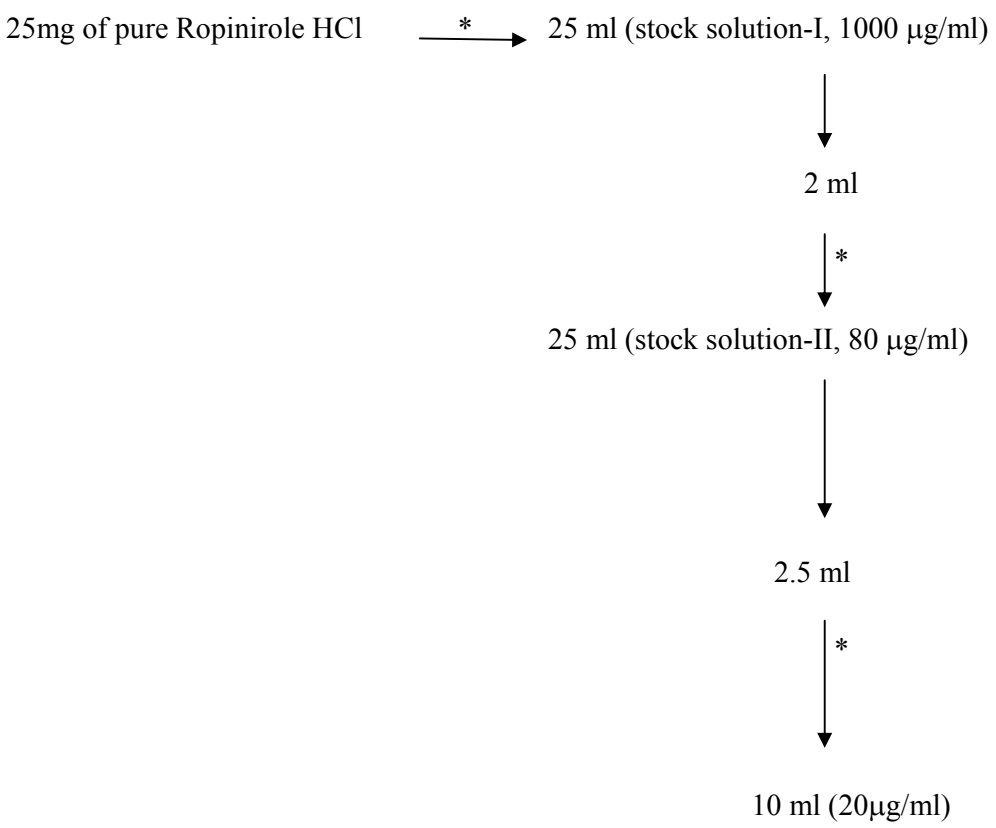


Assay

Preparation of standard solution

From the working standard stock solution – II 2.5 ml was taken and further diluted to 10ml with same solvent to get the final concentration of 20µg/ml. The absorbance of final solution was measured at 250nm against the solvent blank.

Dilution chart



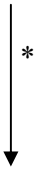
* 0.1 M Acetic acid

Preparation of test solution

Twenty tablets (2 mg) of ropinirole hydrochloride were accurately weighed and grounded to fine powder. Ropinirole hydrochloride tablet powder equivalent to the label claim was accurately weighed and dissolved in 0.1 M acetic acid using vortex mixer and then the solution was diluted to 100ml to get a final concentration of 20µg/ml using same solvent. Then the final solution was filtered and the absorbance of resulting solution was measured at 250nm against the solvent blank.

Dilution chart

2 mg equivalent of ropinirole HCl tablet powder



100 ml (20 µg/ml)

* 0.1 M Acetic acid

Calculation:

Using the absorbance of the standard and sample solution, the content of tablet was calculated as follows.

The content of ropinirole HCl present in each tablet of average weight =

$$\frac{Test\ abs}{Std\ abs} \times \frac{wt\ of\ std}{25} \times \frac{2}{25} \times \frac{2.5}{25} \times \frac{100}{wt\ of\ tab\ powder} \times avg\ wt\ of\ tab \times salt\ factor$$

Table no: 5 Quantitative estimation of Brand I - Ropark

Label claim - 2 mg **Average weight - 0.1431 g**

S.No	Wt of std drug (g)	Abs. of std	Wt of tab. powder (g)	Abs. of test	Amount of drug/tab (mg)	% Label claim
1	0.0245	0.631	0.1196	0.615	2.004	100.20 %
2	0.0245	0.631	0.1283	0.656	1.993	99.65 %
3	0.0245	0.631	0.1352	0.697	2.010	100.50 %
4	0.0245	0.631	0.1380	0.714	2.017	100.85 %
5	0.0245	0.631	0.1298	0.665	1.997	99.85 %

Table no: 6 Quantitative estimation of Brand II- Ropin

Label claim - 2 mg **Average weight - 0.1375 g**

S.No	Wt of std drug (g)	Abs. of std	Wt of tab. powder (g)	Abs. of test	Amount of drug/tab (mg)	% Label claim
1	0.0248	0.642	0.1372	0.769	2.040	102 %
2	0.0248	0.642	0.1334	0.749	2.067	103.35 %
3	0.0248	0.642	0.1428	0.781	2.013	100.65 %
4	0.0248	0.642	0.1390	0.776	2.055	102.75 %
5	0.0248	0.642	0.1370	0.762	2.048	102.40 %

Interference studies

The interference studies of additives used in the formulation of tablet were done by distributing them individually in 0.1 M acetic acid and set aside for ten minutes before filtering. The filtrate was diluted and preceded as per tablet assay. The absorbance of resulting solution was measured at 250nm against the solvent blank.

This procedure was repeated five times of each additive and the average value for each additive was given the following table no. 7

Table no: 7 Data for the interference studies

Name of the excipients	Absorbance at 250 nm
Talc	0.001
Lactose	0.002
Starch	0.001
Mg. Sterate	0.003

The results show that excipients have no effect in the absorption characteristics of the drug.

Recovery studies (Accuracy)

Procedure

The tablet powder equivalent to about 1 mg of ropinirole hydrochloride was weighed accurately and transfer to 100 ml volumetric flask. A known volume of standard stock solution of 1 mg/ml of pure ropinirole hydrochloride was added to the volumetric flask and 30 ml of 0.1 M acetic acid was added and mixed thoroughly using vortex mixer. Then it was diluted to 100 ml using same solvent and it was filtered and absorbance of final solution was measured at 250 nm against the solvent blank.

The percentage recovery was calculated and the procedure was repeated five times for each brand of tablet powder. The result of the recovery studies were given in the table no.8 & 9

Dilution chart

1 mg equivalent of ropinirole HCl tablet

* 1ml of 1mg/ml std drug stock solution

* 0.1 M acetic acid

↓

100 ml (20 μg/ml)

Table no: 8 Recovery studies for brand I - Ropark

S.No	Wt of tab. powder (g)	Amt of std drug added (mg/ml)	Absorbance	Amt recovered (mg)	% recovery
1	0.07130	0.992	0.676	0.978	98.58 %
2	0.07092	0.992	0.671	0.968	97.58 %
3	0.07395	0.992	0.691	0.984	99.19 %
4	0.07254	0.992	0.685	0.986	99.39 %
5	0.07084	0.992	0.669	0.963	97.07 %

Table no: 9 Recovery studies for brand II - Ropin

S.No	Wt of tab. powder (g)	Amt of std added (mg/ml)	Absorbance	Amt recovered (mg)	% recovery
1	0.07081	0.980	0.711	0.961	98.07 %
2	0.06985	0.980	0.702	0.953	97.24 %
3	0.07158	0.980	0.719	0.972	99.18 %
4	0.07237	0.980	0.723	0.971	99.08 %
5	0.07084	0.980	0.709	0.954	97.34 %

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample under prescribed condition.

It is usually expressed as standard deviation. It is a measure of degree of reproducibility of analytical method under normal operation.

Standard deviation (SD) = $\sqrt{\frac{\Sigma(x-\bar{x})^2}{n-1}}$

Where,

SD – Standard deviation

x – Observed value

$\bar{x}$ - Arithmetic mean

n – Number of observations

The square of standard deviation is called variance. The precision is assessed by co-efficient of variation which is related to standard deviation.

Co-efficient of variation (% RSD) = $\frac{SD}{\bar{x}} x\ 100$

Where,

SD – standard deviation

$\bar{x}$ - Arithmetic average

RSD – relative standard deviation

Standard Error of Mean (SEM) = $\sigma_M = \frac{\sigma}{\sqrt{N}}$

Where,

σ_M - Standard error of the mean

σ - The standard deviation of the original distribution

N - The sample size

$\sqrt{N}$ - Root of the sample size

The precision study of this method was done by the data obtained from the assay results shown in the table. 10

Table no: 10 Data for precision study

S.No	Name of tablet	Std deviation	Co-efficient of variation (% RSD)	SEM
1	Ropark	0.4840	0.4829	0.2164
2	Ropin	1.013	0.9909	0.4529

5.2 Extractive Spectrophotometric Determination of Ropinirole hydrochloride using 0.2 % Picric acid

Apparatus/Instruments Used

UV- Visible double beam Spectrophotometer (Perkin Elmer EZ 301)

Analytical electronic weighing balance (Shimadzu)

Vortex mixer

Solvents and Reagents Used

Acetic acid - AR Grade

Chloroform - AR Grade

Picric acid - AR Grade

Water - Distilled Water

Reference Standard

Ropinirole hydrochloride was obtained as a gift sample from East West Pharma Haridwar - 247 667, India.

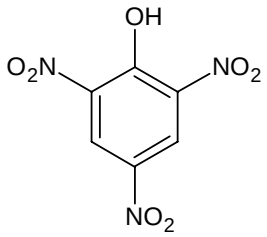
Tablet Formulations

Ropark film coated tablets – 2 mg (Sun Pharmaceuticals Industries Ltd)

Ropin film coated tablets – 2 mg (East West Pharm

Chemistry of Picric acid

Chemical Structure



CAS Registry Number - 88-89-1

Appearance - Bright yellow, crystalline powder or yellow prisms

IUPAC Name - 2, 4, 6 - Trinitrophenol

Molecular Formula - $C_6H_3N_3O_7$

Molar Mass - 229.11 g mol^{-1}

Solubility - Soluble in hot water

Preparation of 0.1 M Acetic acid

It was prepared by diluting 5.7 ml of concentrated acetic acid in 1000 ml of distilled water.

Preparation of 1 % Potassium hydrogen phthalate

It was prepared by dissolving 1 g of potassium hydrogen phthalate in 100 ml of distilled water.

Preparation of 0.2 % Picric acid

It was prepared by dissolving 0.2 g of picric acid in 100 ml of hot distilled water

Preparation of standard stock solution

Standard stock solution - I

Standard stock solution - I was prepared by dissolving 40 mg of ropinirole hydrochloride working standard in 100 ml of 0.1 M acetic acid to get a final concentration of 400 µg/ml.

Standard stock solution - II

From the standard stock solution - I 5 ml was taken and further diluted to 50ml with same solvent to get the final concentration of 40 µg/ml.

Absorption spectra of colored species

2 ml of standard stock solution II was pipetted out and transferred in to a separating funnel followed by the addition of 2 ml of 0.2 % picric acid was added. The drug dye complex in aqueous phase was extracted with two successive quantities of chloroform. The combined chloroform extract was diluted to 10 ml with chloroform. The absorption spectra of the final solution were scanned in the range of 300 to 500 nm against the reagent blank. The absorbance data were presented in table no. 11 and absorption spectrum was shown in figure no. 6

Table no: 11 Data for the absorption spectrum of ropinirole hydrochloride using 0.2 % picric acid

S.No	Wavelength	Absorbance
1	300	0.115
2	310	0.155
3	320	0.224
4	330	0.302
5	340	0.359
6	350*	0.361
7	360	0.302
8	370	0.228
9	380	0.183
10	390	0.171
11	400	0.175
12	410*	0.175
13	420	0.163
14	430	0.126
15	440	0.075
16	450	0.034
17	460	0.012
18	470	0.004
19	480	0.002
20	490	0.002
21	500	0.002

Figure no: 6 Absorption spectrum of ropinirole hydrochloride using 0.2 % picric acid

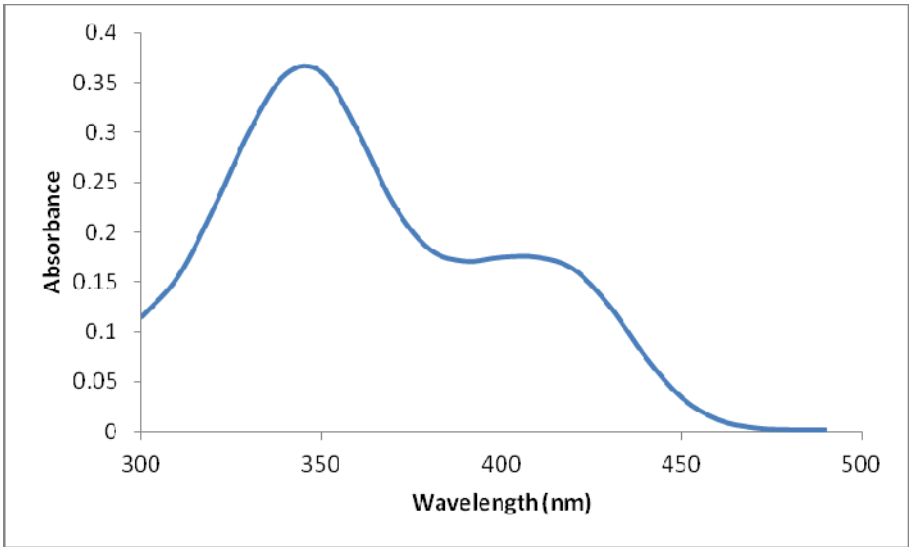


Table no: 12 System Parameters

Wavelength Scan	
Data Mode	Absorbance
Start WL (nm)	500.00
Stop WL (nm)	300.00
Scan Speed (nm/min)	100
Slit Width (nm)	1.5
Path Length (nm)	10.0

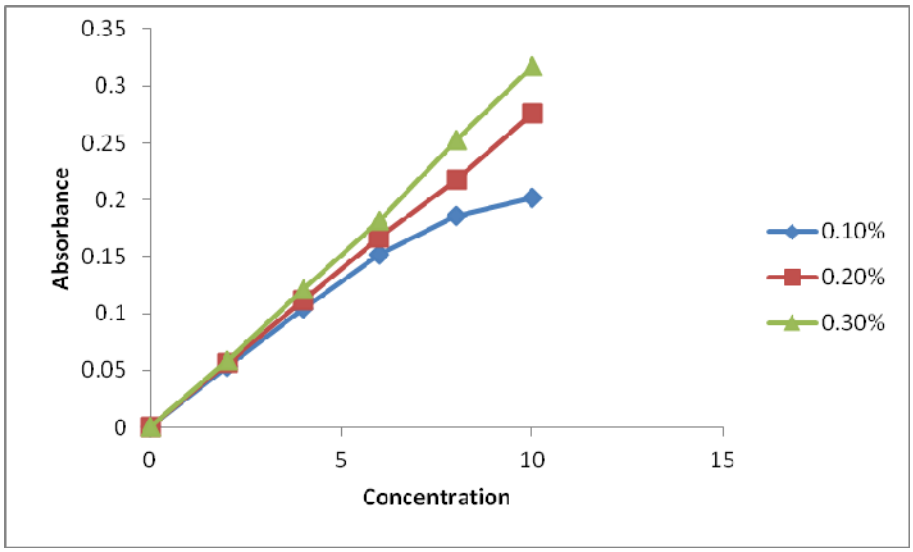
Effect of reagent concentration

Choosing of correct reagent concentration is a significant characteristic criterion in the colorimetric determination. The optimal concentration was predetermined by constructing the calibration curve. The calibration curve was constructed by treating the drug in the concentration range of 2 - 10 µg/ml with 2 ml of different concentration of reagent i.e. 0.1 %, 0.2 %, 0.3 % picric acid. The readings were tabulated in the table no: 13 and graphically shown in the figure no: 7

Table no: 13 Data for calibration curve using different reagent concentration

Drug concentration (µg/ml)	**Absorbance at 410 nm**		
	0.1 %	**0.2 %**	**0.3 %**
2	0.053	0.057	0.059
4	0.104	0.112	0.121
6	0.152	0.167	0.182
8	0.186	0.218	0.253
10	0.202	0.276	0.317

Figure no: 7 Calibration curve for ropinirole hydrochloride using different concentration of picric acid regent



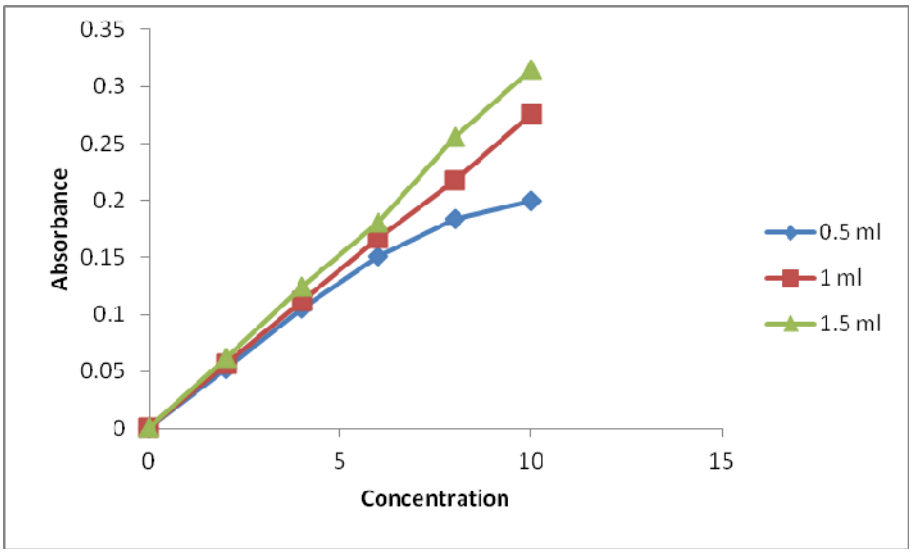
Effect of volume of reagent

Addition of correct volume of reagent was an important aspect in this experiment. The optimum amount of reagent was fixed by constructing calibration curve. The calibration curve was prepared by employing the drug in the concentration range of 2-10 μg/ml with different volume of reagent i.e. 1 ml, 2 ml, 3ml with same reagent concentration (0.2 % picric acid). The results were recorded in table no. 14 and graphically shown in figure no. 8

Table no: 14 Data for the calibration curve for drug with different volume of 0.2 % picric acid reagent

Drug Concentration (μg/ml)	Absorbance at 410 nm		
	0.5 ml	**1 ml**	**1.5 ml**
2	0.052	0.057	0.062
4	0.105	0.112	0.124
6	0.151	0.167	0.180
8	0.184	0.218	0.256
10	0.200	0.276	0.315

Figure no: 8 Calibration curve for ropinirole hydrochloride using different volume of 0.2 % picric acid regent



Fixation of various parameters

I) λmax (wavelength maximum)

The absorption spectral data showed that the maximum absorbance was observed at 350 and 410 nm.

II) Construction of calibration curve

Aliquots of working standard stock solution - II ranging from (0.5 ml to 2.5 ml) were transferred into a series of separating funnel followed by the addition of 2 ml of 0.2 % picric acid. The drug dye complex in aqueous phase was extracted with two successive quantities of chloroform. The combined chloroform extract was diluted to 10 ml with chloroform. The absorbance of the final solution was measured at 410 nm against the reagent blank. Calibration curve was constructed by plotting absorbance vs. concentration. Data for calibration curve were presented in table no.15 and calibration curve was shown in figure no.9

Dilution chart of calibration curve

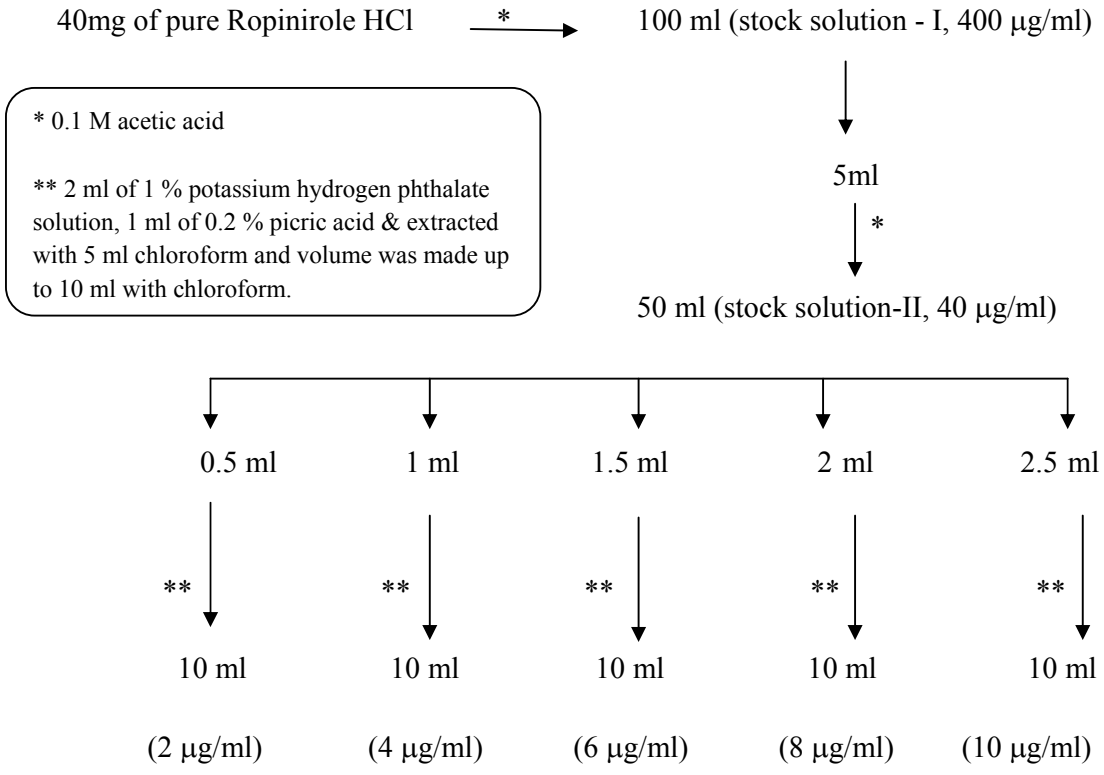
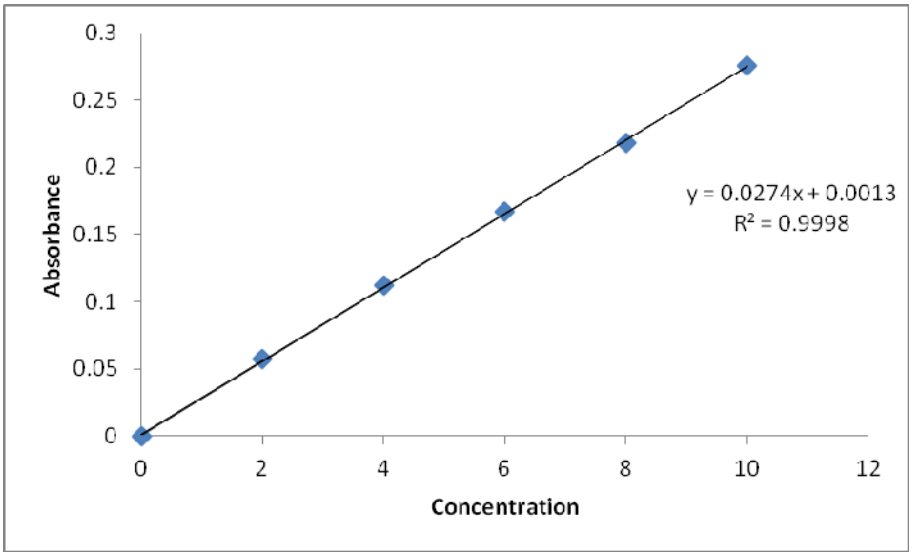


Table no: 15 Data for calibration curve of ropinirole hydrochloride using 0.2 % picric acid reagent

Concentration (µg/ml)	Absorbance
2	0.057
4	0.112
6	0.167
8	0.218
10	0.276

Figure no: 9 Calibration curve of ropinirole hydrochloride using 0.2 % picric acid reagent



IV) Stability of color

The stability of the color is one of the aspects that should be considered in colorimetric determination. The color should be stable for reasonable time.

The color stability of the drug - picric acid complex was studied for the drug concentration in the range of 2 - 10 μg/ml. The absorption of the final solution was measured at 410 nm against reagent blank. The readings were taken at 10 minutes intervals of time up to 60 minutes and results were tabulated in the table no.15

Table no: 16 Data for color stability of drug-picric acid complex

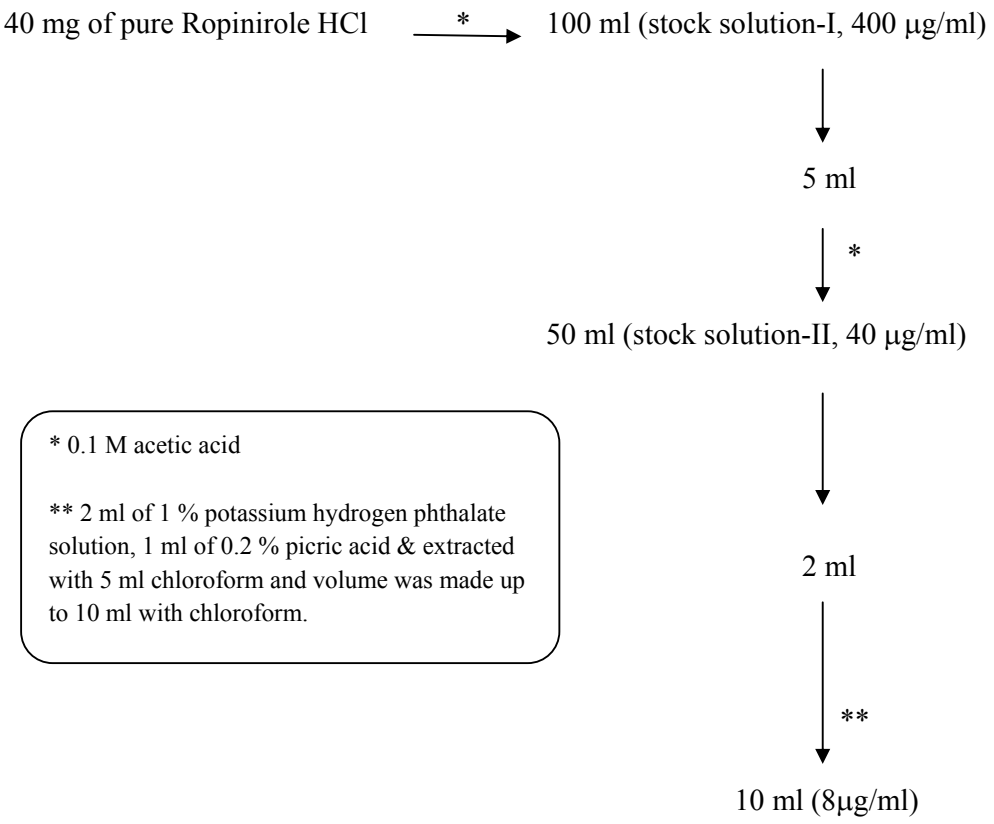
Drug conc. (μg/ml)	Time and Absorption (minutes)						
	0	**10**	**20**	**30**	**40**	**50**	**60**
2	0.057	0.057	0.055	0.058	0.058	0.061	0.061
4	0.112	0.112	0.111	0.111	0.113	0.114	0.113
6	0.167	0.169	0.168	0.167	0.168	0.170	0.169
8	0.218	0.218	0.217	0.217	0.219	0.220	0.220
10	0.276	0.275	0.277	0.279	0.278	0.281	0.281

Assay

Preparation of standard solution

From the working standard stock solution – II 2 ml was taken and transferred in to a separating funnel followed by the addition of 2 ml of 1 % potassium hydrogen phthalate solution and 1 ml of 2 % picric acid solution. The drug-picric acid complex was extracted with two successive quantities of chloroform. Then the combined chloroform extract was further diluted to 10ml with chloroform to get the final concentration of 8 μg/ml. The absorbance of final solution was measured at 410 nm against the reagent blank.

Dilution chart



Preparation of test solution

Twenty tablets (1 mg) of ropinirole hydrochloride were accurately weighed and grounded to fine powder and dissolved in 0.1 M acetic acid using vortex mixer. Then the solution was diluted to 25 ml to get a drug concentration of 40µg/ml using 0.1 M acetic acid and resulting solution was filtered to remove excipients. From this 2 ml was transferred in to a separating funnel followed by the addition of 2 ml of 1 % potassium hydrogen phthalate solution and 1 ml of 2 % picric acid. The drug-picric acid complex was extracted by using two successive quantities of chloroform. The combined chloroform extract was collected in a 10 ml volumetric flask and volume was made up with chloroform. Finally the absorbance of resulting solution was measured at 410nm against the reagent blank.

Dilution chart

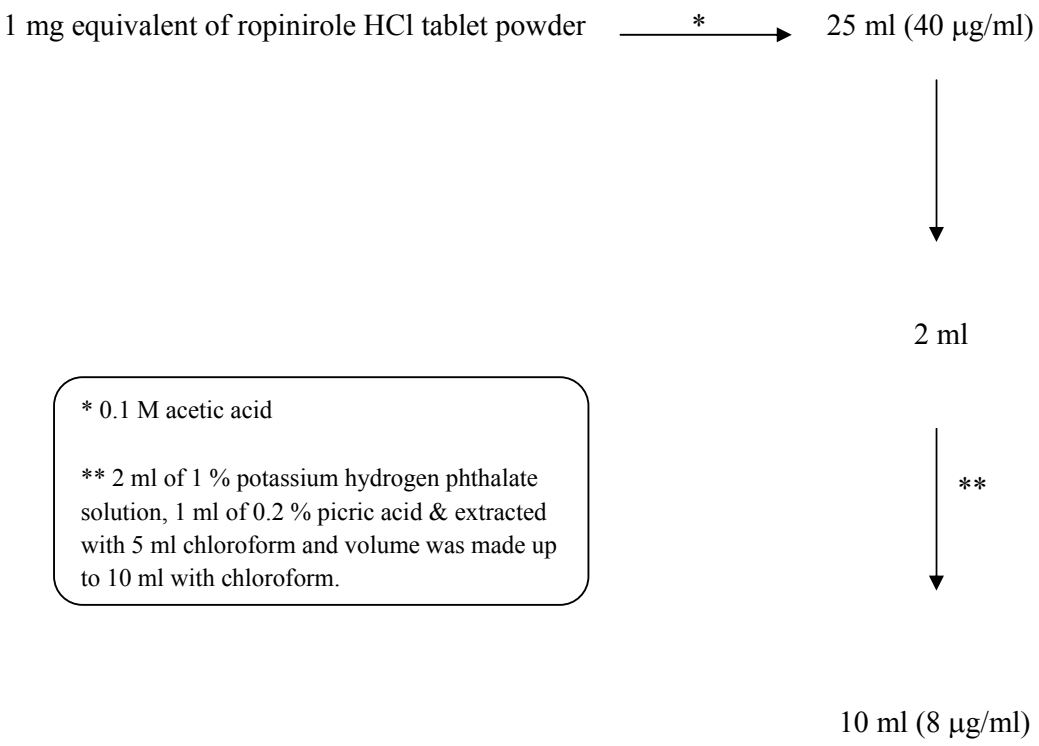


Table no: 17 Quantitative estimation of Brand I - Ropark

Label claim - 2 mg **Average weight - 0.1431 g**

S.No	Wt of std drug (g)	Abs. of std	Wt of tab. powder (g)	Abs. of test	Amount of drug/tab (mg)	% Label claim
1	0.0402	0.215	0.0695	0.239	2.017	100.85 %
2	0.0402	0.215	0.0681	0.235	2.024	101.20 %
3	0.0402	0.215	0.0693	0.236	1.998	99.90 %
4	0.0402	0.215	0.0704	0.238	1.983	99.15 %
5	0.0402	0.215	0.0691	0.237	2.012	100.60 %

Table no: 18 Quantitative estimation of Brand II- Ropin

Label claim - 2 mg **Average weight - 0.1375 g**

S.No	Wt of std drug (g)	Abs. of std	Wt of tab. powder (g)	Abs. of test	Amount of drug/tab (mg)	% Label claim
1	0.0404	0.219	0.0656	0.235	1.996	99.80 %
2	0.0404	0.219	0.0675	0.238	1.961	98.05 %
3	0.0404	0.219	0.0649	0.232	1.988	99.40 %
4	0.0404	0.219	0.0653	0.232	1.976	98.80 %
5	0.0404	0.219	0.0687	0.241	1.951	97.55 %

Interference studies

The interference studies of additives used in the formulation of tablet were done by distributing them individually in 0.1 M acetic acid and set aside for ten minutes before filtering. The filtrate was diluted and preceded as per tablet assay. The absorbance of resulting solution was measured at 410nm against the reagent blank.

This procedure was repeated five times of each additive and the average value for each additive was given the following table.

Table no: 19 Data for the interference studies

Name of the excipients	Absorbance at 410 nm
Talc	0.003
Lactose	0.001
Starch	0.002
Mg. Sterate	0.003

The results show that excipients have no effect in the absorption characteristics of the drug.

Recovery studies (Accuracy)

Procedure

The tablet powder equivalent to about 1 mg of ropinirole hydrochloride was weighed accurately and transfer to 50 ml volumetric flask. A known volume of standard stock solution of 1 mg/ml of pure ropinirole hydrochloride was added to the volumetric flask and 15 ml of 0.1 M acetic acid was added and mixed thoroughly using vortex mixer. Then it was diluted to 50 ml using 0.1 M acetic acid and it was filtered. 2 ml of filtrate was transferred in to a separating funnel. The absorbance of final solution was measured at 410 nm against the reagent blank.

The percentage recovery was calculated and the procedure was repeated five times for each brand of tablet powder. The result of the recovery studies was given in the table no. 20 & 21

Dilution chart

1 mg equivalent of ropinirole HCl tablet powder + 1 ml of 1 mg/ml std stock soln

↓ *

50 ml (40 µg/ml)

↓

2 ml

↓ **

10 ml (8 µg/ml)

* 0.1 M acetic acid

** 2 ml of 1 % potassium hydrogen phthalate solution, 1 ml of 0.2 % picric acid & extracted with 5 ml chloroform and volume was made up to 10 ml with chloroform.

Table no: 20 Recovery studies for brand I - Ropark

S. No	Wt of tab. powder	Amt of std added (mg/ml)	Absorbance	Amt recovered (mg)	% recovery
1	0.0734	1.012	0.229	1.006	99.40 %
2	0.0715	1.012	0.226	1.007	99.59 %
3	0.0746	1.012	0.228	0.978	96.64 %
4	0.0718	1.012	0.224	0.984	97.23 %
5	0.0749	1.012	0.232	1.011	99.90 %

Table no: 21 Recovery studies for brand II - Ropin

S. No	Wt of tablet powder	Amt of std added (mg/ml)	Absorbance	Amt recovered (mg)	% recovery
1	0.0687	0.994	0.222	0.953	95.89 %
2	0.0678	0.994	0.221	0.976	98.18 %
3	0.0665	0.994	0.218	0.968	97.38 %
4	0.0696	0.994	0.226	0.993	99.89 %
5	0.0675	0.994	0.221	0.990	99.59 %

Precision

The precision study of this method was done by the data obtained from the assay results shown in the table no. 22

Table no: 22 Data for precision

S. No.	Name of tablet	Std deviation	Co-efficient of variation (% RSD)	SEM
1	Ropark	0.8181	0.8153	0.3659
2	Ropin	0.9291	0.9411	0.4155

5. 3 RP-HPLC Method for the Determination of Ropinirole hydrochloride

Apparatus/Instruments Used

HPLC Separation Module (Perkin Elmer)

Analytical electronic weighing balance (Shimadzu)

Vortex Mixer

Sonicator

Micropipette

pH meter

Membrane filter

Solvents and Reagents Used

Water - HPLC grade

Potassium dihydrogen phosphate - HPLC grade

Acetonitrile - HPLC grade

Reference Standard

Ropinirole hydrochloride was obtained as a gift sample from East West Pharma Haridwar - 247 667, India.

Tablet Formulations

Ropark film coated tablets - 2 mg (Sun Pharmaceuticals Industries Ltd)

Ropark film coated tablets - 2 mg (East West Pharma)

Preparation of 20 mM potassium dihydrogen phosphate buffer

It is prepared by dissolving 2.7218 g in 1000 ml of HPLC grade water and the pH was adjusted to 3.3 with 10 % ortho phosphoric acid.

Preparation of mobile phase

Mobile phase was prepared by mixing 70:30 ratio volumes of 20 mM KH_2PO_4: ACN. This mobile phase was filtered through 0.45 µ membrane filter paper and degassed in ultrasonicator bath for 10 minutes

Preparation standard stock solutions

Standard stock solution I

Standard stock solution - I was prepared by dissolving 10 mg of ropinirole hydrochloride working standard in 10 ml of mobile phase mixture to get a final concentration of 1000 µg/ml.

Standard stock solution II

From the standard solution - I, 1 ml was taken and further diluted to 10 ml with mobile phase mixture to get the final concentration of 100 µg/ml.

Selection of stationary phase

From the literature survey, it was found that octadecyl silane column (C_{18}) can be appropriately used for the separation of ropinirole hydrochloride.

Selection of analytical wavelength

From the UV spectrum of the compound, the maximum absorbance of Ropinirole hydrochloride was found to be 250 nm and this wavelength was suitable for detection. So the appreciable absorbance for the drug was found at 250 nm.

CHROMATOGRAPHIC CONDITIONS:

Optimized conditions for the determination of Ropinirole hydrochloride by developed HPLC method was shown as follows:

Column	: Kromosil $C_{18,}$ [5µ, 250 x 4.60 mm]
Mobile phase	: 20 mM KH_2PO_4: ACN (70: 30) pH 3.3
Flow rate	: 1 ml/min
Detector	: UV detector
Injection volume	: 20 µl
Run time	: 7.5 min
Mode of elution	: Isocratic
Detection Wavelength	: 250 nm

Construction of linearity

The linear concentration range was established for the drug solution in the concentration range of 4 - 16 µg/ml of ropinirole hydrochloride. 0.4 - 1.6 ml of stock solution II was pipetted in to 10 ml volumetric flask and diluted to 10 ml to get the desired concentration range. The calibration curve for ropinirole hydrochloride was constructed by plotting the peak area against the concentration. Correlation coefficient (r), y intercept, slope of regression line was calculated. The recorded data were shown in the table no: 22 and linearity graph was shown in the figure no: 10

Dilution chart

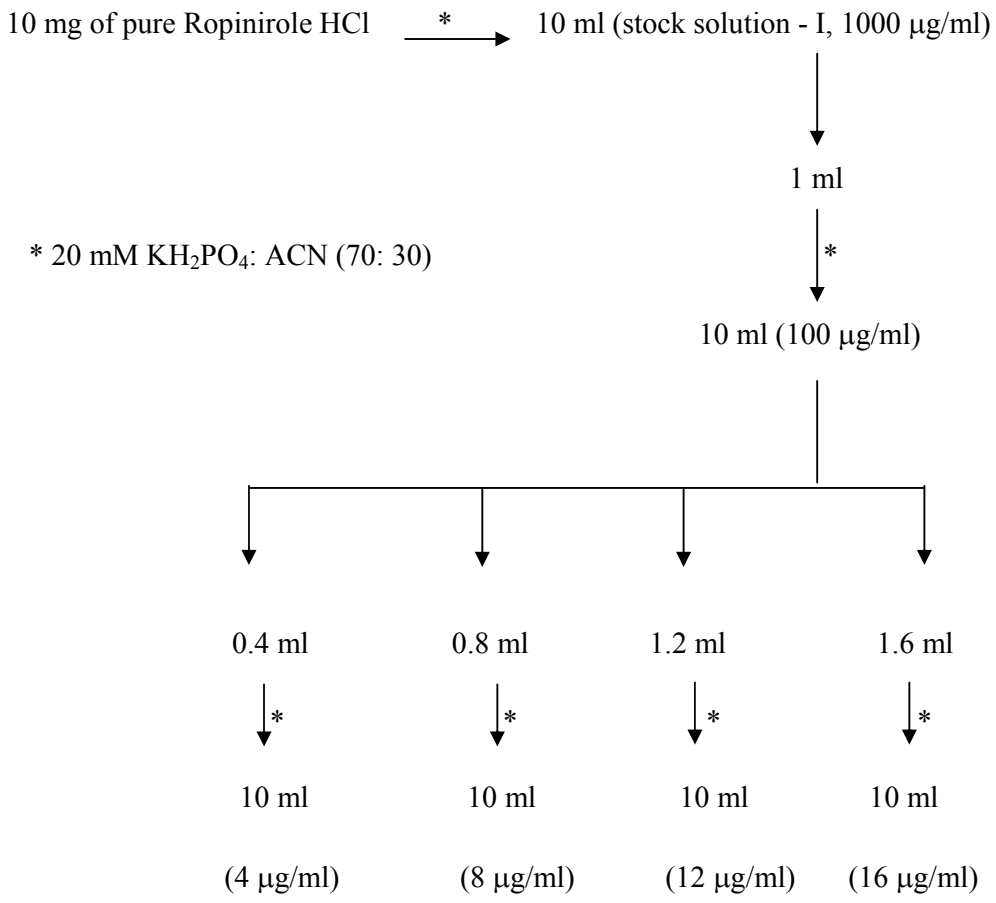
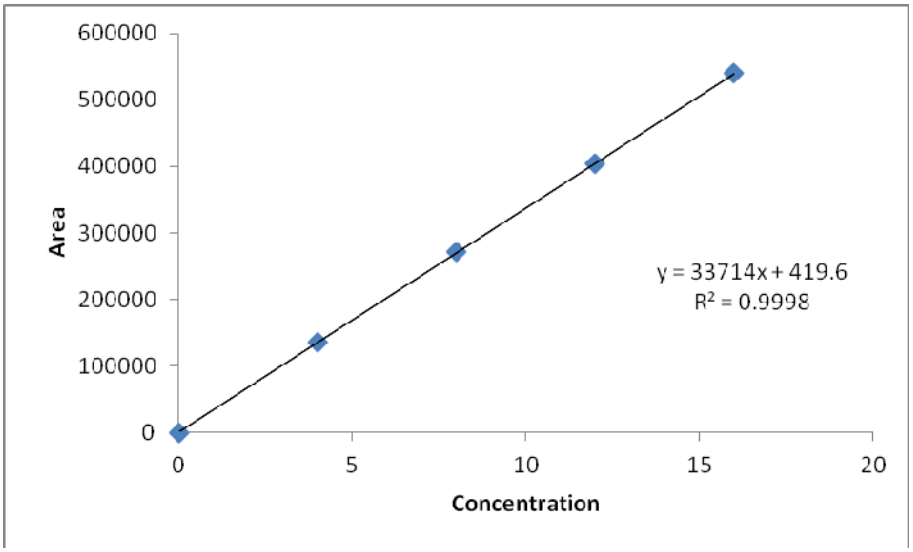


Table no: 23 Data for linearity curve for Ropinirole hydrochloride

S.No	Conc. (µg/ml)	Peak area
1	4	135505.22
2	8	270912.44
3	12	404413.65
4	16	539824.88

Figure no: 10 Linearity curve of Ropinirole hydrochloride



Chromatogram: 1

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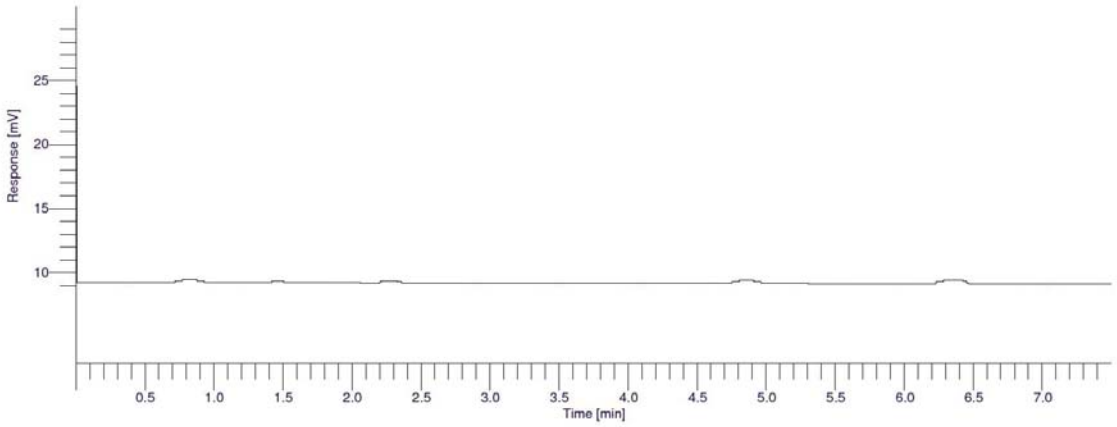
Operator	: manager	Sample Name	: Blank
Sample Number	: 001	Study	:
AutoSampler	: NONE	Rack/Vial	: 0/0
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 7.50 min
Sampling Rate	: 10.0000 pts/s		
Sample Volume	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 12/15/2011 2:02:34 PM	Cycle	: 2



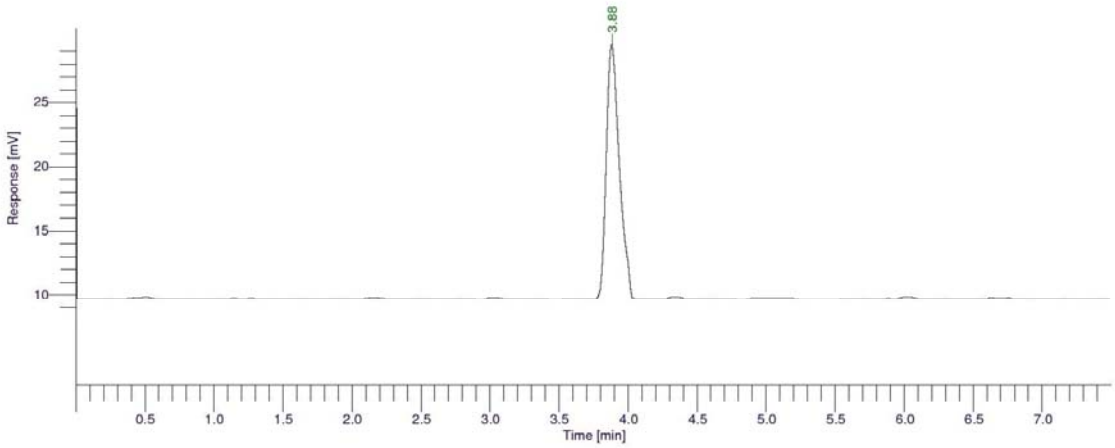
ROPINIROLE HCl

K.M.COLLEGE OF PHARMACY

Peak #	Component Name	Time [min]	Area [uV*sec]	Height [uV]	Area [%]
1		0.815	172.14	32.93	23.42
2		1.482	98.54	17.86	12.70
3		2.259	156.24	28.54	20.29
4		4.783	124.85	31.52	22.41
5		6.429	139.24	29.75	21.15
			691.01	140.60	100.00

Chromatogram: 2

Software Version	: 6.3.0.0445	Date	: 12/15/2011
Operator	: manager	Sample Name	: Ropinirole HCl 4mcg/ml
Sample Number	: 002	Study	:
AutoSampler	: NONE	Rack/Vial	: 0/0
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 7.50 min
Sampling Rate	: 10.0000 pts/s		
Sample Volume	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 12/15/2011 2:24:34 PM	Cycle	: 4



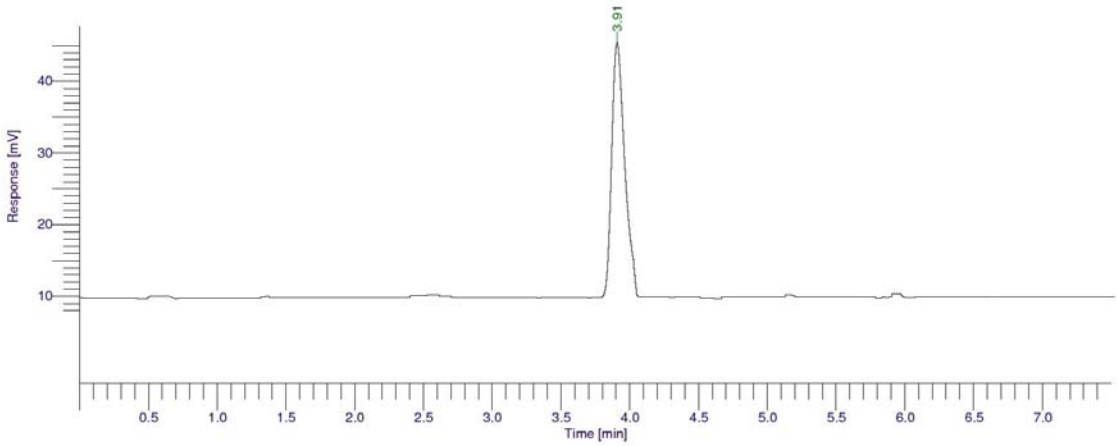
ROPINIROLE HCl

K.M.COLLEGE OF PHARMACY

Peak #	Component Name	Time [min]	Area [uV*sec]	Height [uV]	Area [%]
1		0.489	61.05	26.24	0.04
2		1.115	26.87	17.23	0.01
3		2.225	34.93	19.56	0.02
4		3.105	32.16	18.98	0.02
5		3.877	135505.22	22642.75	99.75
6		4.354	58.46	24.42	0.04
7		6.012	95.35	37.60	0.07
8		6.610	24.15	16.20	0.01
			135838.19	22802.98	100.00

Chromatogram: 3

Software Version	: 6.3.0.0445	Date	: 12/15/2011
Operator	: manager	Sample Name	: Ropinirole HCl 8mcg/ml
Sample Number	: 003	Study	:
AutoSampler	: NONE	Rack/Vial	: 0/0
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 7.50 min
Sampling Rate	: 10.0000 pts/s		
Sample Volume	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 12/15/2011 2:32:33 PM	Cycle	: 5



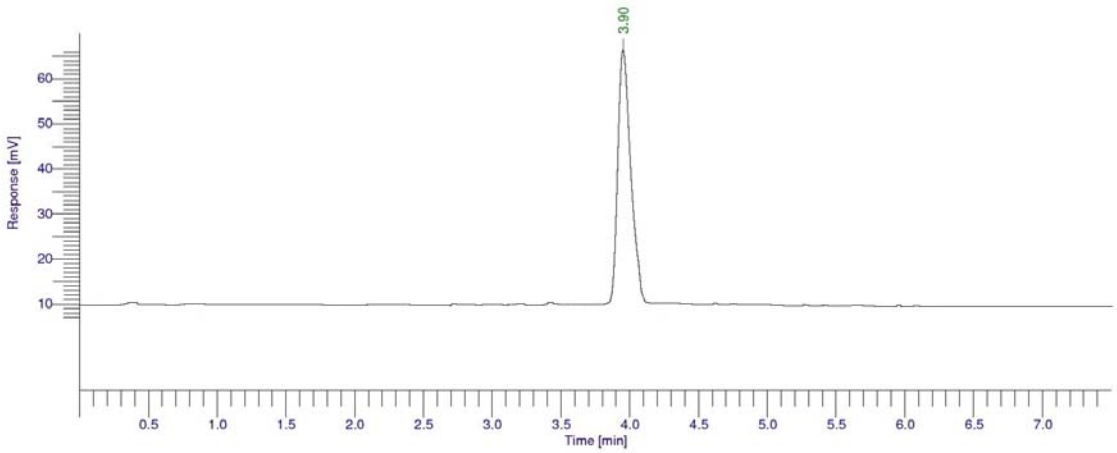
ROPINIROLE HCl

K.M.COLLEGE OF PHARMACY

Peak #	Component Name	Time [min]	Area [uV*sec]	Height [uV]	Area [%]
1		0.621	55.51	21.02	0.02
2		1.392	92.42	44.46	0.03
3		2.626	84.59	32.90	0.03
4		3.909	270912.44	45705.34	99.88
5		5.215	43.61	19.80	0.01
6		5.967	44.92	20.07	0.01
			271233.49	45843.59	100.00

Chromatogram: 4

Software Version	: 6.3.0.0445	Date	: 12/15/2011
Operator	: manager	Sample Name	: Ropinirole HCl 12mcg/ml
Sample Number	: 004	Study	:
AutoSampler	: NONE	Rack/Vial	: 0/0
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 7.50 min
Sampling Rate	: 10.0000 pts/s		
Sample Volume	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 12/15/2011 2:40:34 PM	Cycle	: 6



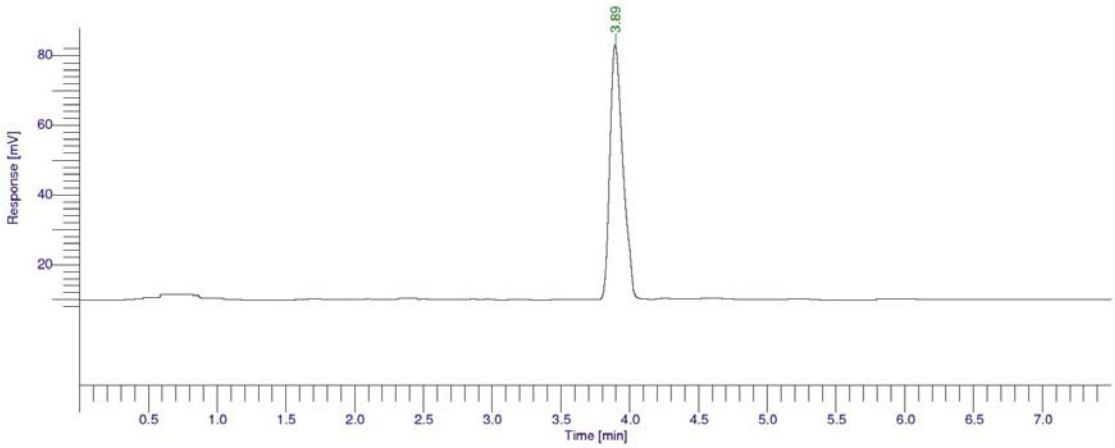
ROPINIROLE HCL

K.M.COLLEGE OF PHARMACY

Peak #	Component Name	Time [min]	Area [uV*sec]	Height [uV]	Area [%]
1		0.394	46.75	24.43	0.01
2		2.725	32.41	18.63	0.01
3		3.243	31.33	18.22	0.01
4		3.498	34.64	19.02	0.01
5		3.902	404413.65	60462.29	99.93
6		4.611	18.44	10.12	0.01
7		5.321	21.22	13.62	0.01
8		5.912	19.12	12.02	0.01
			404617.57	60578.35	100.00

Chromatogram: 5

Software Version	: 6.3.0.0445	Date	: 12/15/2011 2:48:34 PM
Operator	: manager	Sample Name	: Ropinirole HCl 16mcg/ml
Sample Number	: 005	Study	:
AutoSampler	: NONE	Rack/Vial	: 0/0
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 7.50 min
Sampling Rate	: 10.0000 pts/s	Area Reject	: 0.000000
Sample Volume	: 1.000000 ul	Dilution Factor	: 1.00
Sample Amount	: 1.0000	Cycle	: 7
Data Acquisition Time	: 12/15/2011 2:48:34 PM		



ROPINIROLE HCL

K.M.COLLEGE OF PHARMACY

Peak #	Component Name	Time [min]	Area [uV*sec]	Height [uV]	Area [%]
1		0.732	94.68	64.09	0.02
2		1.694	29.06	18.43	0.01
3		2.521	38.42	22.64	0.01
4		2.996	24.23	17.92	0.01
5		3.890	539824.88	74265.03	99.93
6		4.312	32.64	21.23	0.01
7		5.256	31.23	21.02	0.01
			540075.14	74430.36	100.00

ASSAY

Preparation of standard solution

From the working standard solution - II, 0.8 ml was pipetted out in to a 10 ml volumetric flask and the volume was made up with mobile phase mixture containing 20 mM KH_2PO_4: ACN (70: 30) to get a final concentration of 8 µg/ml.

20 µl of the above solution was injected in triplicate at an interval of 7.5 min to ensure complete elution of earlier injection. The peak area was measured and recorded.

Dilution chart

10 mg of pure Ropinirole HCl —*→ 10 ml (stock solution - I, 1000 µg/ml)

↓

1 ml

* 20 mM KH_2PO_4: ACN (70: 30) *

↓

10 ml (stock soln - II, 100 µg/ml)

↓

0.8 ml

*

↓

10 ml (8 µg/ml)

Preparation of test solution

Twenty tablets (2 mg) of Ropinirole hydrochloride were accurately weighed and grounded to fine powder. Ropinirole hydrochloride tablet powder equivalent to 1 mg was accurately weighed and dissolved in mobile phase using vortex mixture and the volume was made up to 10 ml with mobile phase to get a concentration of 100 µg/ml. Then this solution was filtered, from this 0.8 ml o0f filtrate was pipetted out into 10 ml of volumetric flask and the volume was made up to 10 ml with mobile phase solution to get a final concentration of 8 µg/ml.

20 µl of the above solution was injected in triplicate at an interval of 7.5 min to ensure complete elution of earlier injection. The peak area was measured and recorded.

Dilution chart

1 mg equivalent of tablet powder $\xrightarrow{*}$ 10 ml (100 µg/ml)

↓

0.8 ml

↓ *

10 ml (8 µg/ml)

* 20 mM KH_2PO_4: ACN (70: 30)

Assay calculation

The content of ropinirole HCl present in each tablet of average weight =

$$\frac{Area\ of\ test}{Area\ of\ std} \times \frac{wt\ of\ std}{wt\ of\ test} \times dilution\ factor \times avg\ wt \times salt\ factor$$

Table no: 24 Quantitative estimation of Brand I - Ropark

Label claim - 2 mg **Average weight - 0.1431 g**

S.No	Standard		Tablet		Amount of drug/tab (mg)	% Label claim
	Wt of Std drug (mg)	Peak area	Wt of tab. powder (mg)	Peak area		
1	9.8	272512.44	70.7	312426.45	2.003	100.15 %
2	9.8	272512.44	71.6	312916.58	1.988	99.06 %
3	9.8	272512.44	70.9	312469.23	1.997	99.85 %
4	9.8	272512.44	71.2	312522.13	1.989	99.45 %
5	9.8	272512.44	68.3	312389.23	2.042	102.10 %

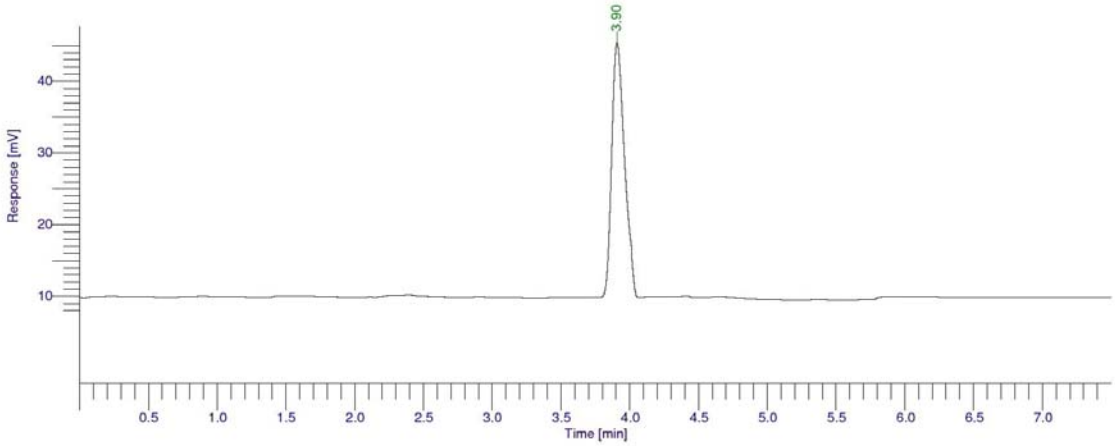
Table no: 25 Quantitative estimation of Brand II- Ropin

Label claim - 2 mg **Average weight - 0.1375 g**

S.No	Standard		Tablet		Amount of drug/tab (mg)	% Label claim
	Wt of Std drug (mg)	Peak area	Wt of tab. powder (mg)	Peak area		
1	9.8	272512.44	66.2	311720.35	2.042	102.10 %
2	9.8	272512.44	67.4	312141.18	2.009	100.45 %
3	9.8	272512.44	65.8	311410.18	2.053	102.65 %
4	9.8	272512.44	67.1	312128.30	2.018	100.90 %
5	9.8	272512.44	66.0	311568.24	2.047	102.35 %

Chromatogram: 6

Software Version	: 6.3.0.0445	Date	: 12/15/2011
Operator	: manager	Sample Name	: Ropinirole HCl Std 8mcg/ml
Sample Number	: 006	Study	:
AutoSampler	: NONE	Rack/Vial	: 0/0
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 7.50 min
Sampling Rate	: 10.0000 pts/s		
Sample Volume	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 12/15/2011 2:57:33 PM	Cycle	: 8



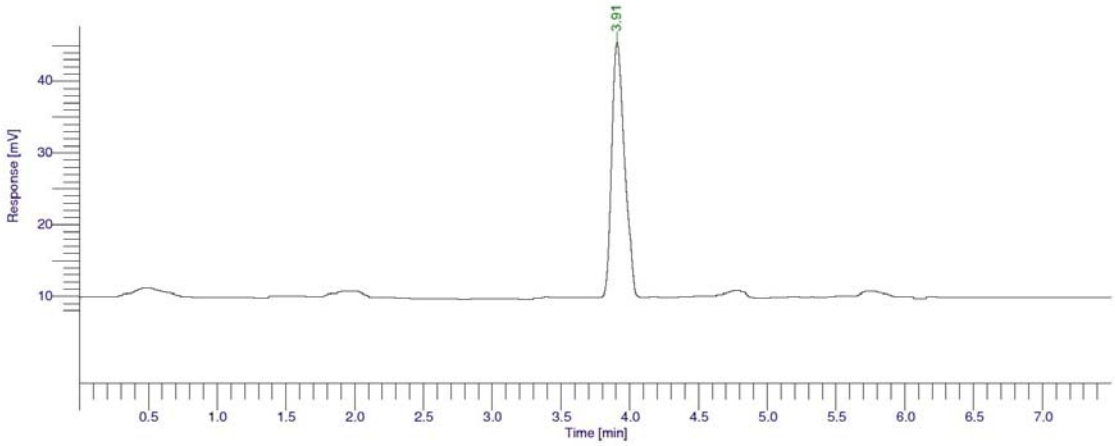
ROPINIROLE HCl

K.M.COLLEGE OF PHARMACY

Peak #	Component Name	Time [min]	Area [uV*sec]	Height [uV]	Area [%]
1		0.201	20.64	12.83	0.01
2		0.985	24.65	14.32	0.01
3		2.468	43.27	28.40	0.01
4		3.901	272512.44	46705.34	99.95
5		4.492	34.62	24.69	0.01
6		5.752	32.43	23.46	0.01
			272668.05	46809.04	100.00

Chromatogram: 7

Software Version	: 6.3.0.0445	Date	: 12/15/2011
Operator	: manager	Sample Name	: Ropark Assay
Sample Number	: 007	Study	:
AutoSampler	: NONE	Rack/Vial	: 0/0
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 7.50 min
Sampling Rate	: 10.0000 pts/s		
Sample Volume	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 12/15/2011 3:05:38 PM	Cycle	: 9



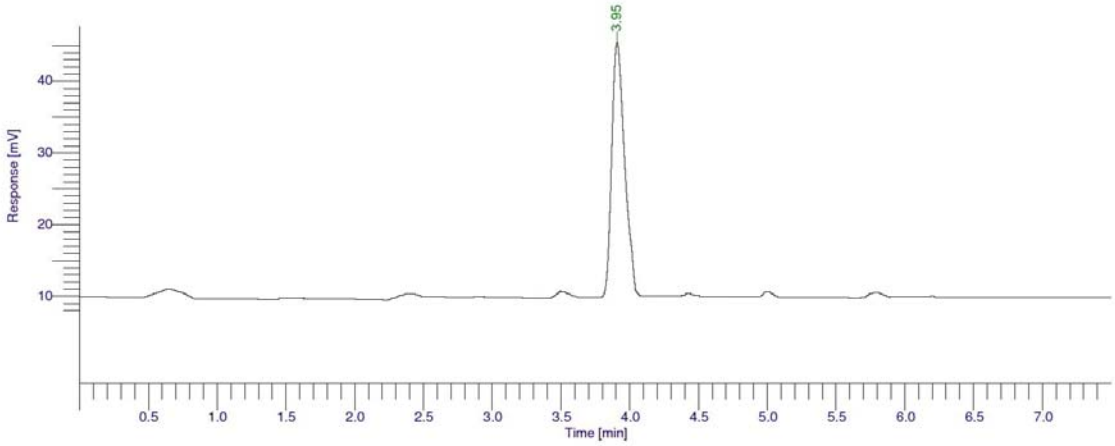
ROPINIROLE HCl

K.M.COLLEGE OF PHARMACY

Peak #	Component Name	Time [min]	Area [uV*sec]	Height [uV]	Area [%]
1		0.496	436.43	191.03	0.13
2		1.982	391.12	168.13	0.12
3		3.495	32.28	21.02	0.01
4		3.911	312426.45	50605.33	99.50
5		4.789	396.12	178.16	0.12
6		5.711	321.56	152.44	0.12
			313971.68	51295.09	100.00

Chromatogram: 8

Software Version	: 6.3.0.0445	Date	: 12/15/2011
Operator	: manager	Sample Name	: Ropin Assay
Sample Number	: 013	Study	:
AutoSampler	: NONE	Rack/Vial	: 0/0
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 7.50 min
Sampling Rate	: 10.0000 pts/s		
Sample Volume	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 12/15/2011 3:58:38 PM	Cycle	: 15



ROPINIROLE HCl

K.M.COLLEGE OF PHARMACY

Peak #	Component Name	Time [min]	Area [uV*sec]	Height [uV]	Area [%]
1		0.689	392.46	169.72	0.12
2		1.510	19.22	10.44	0.01
3		2.498	296.14	141.23	0.11
4		3.504	369.32	161.32	0.12
5		3.951	311720.35	49968.33	99.61
6		4.428	84.36	43.62	0.01
7		5.012	112.67	58.23	0.01
8		5.753	124.43	63.12	0.01
			313118.95	50616.01	100.00

Recovery Studies (Accuracy)

The recovery experiment was performed for 2 brands of tablet. Tablet powder equivalent 0.8 mg of ropinirole hydrochloride was weighed. A known volume of standard solution (0.2 mg/ml) of pure sample of ropinirole hydrochloride was added to the tablet powder. Then the dilution was made similar to assay formulation. The recovery percentage was calculated. It was repeated for 5 times. The recovery studies were shown in table: 26 & 27

Dilution chart

0.8 mg equivalent of tab powder + 1ml of 0.2 mg/ml of pure ropinirole HCl

10 ml (100 µg/ml)

0.8 ml

10 ml (8 µg/ml)

* 20 mM KH_2PO_4: ACN (70: 30)

Table no: 26 Recovery studies for brand I - Ropark

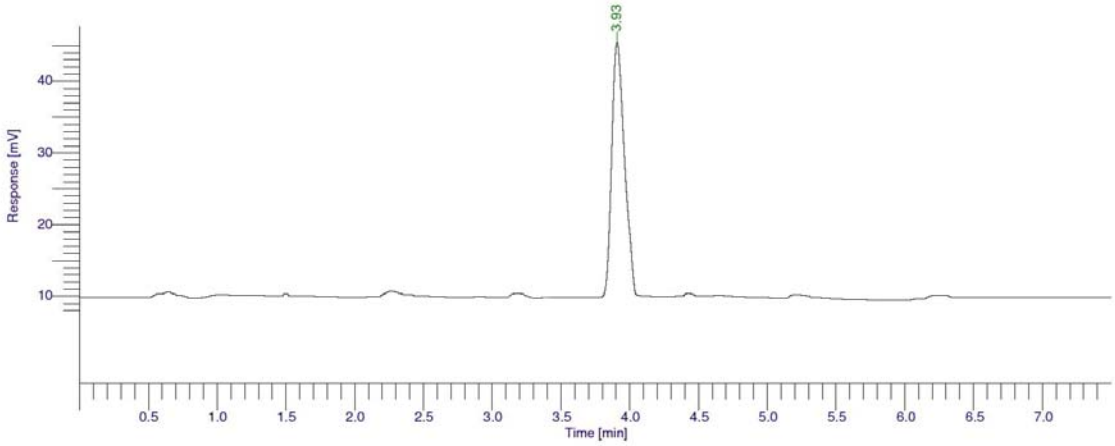
S.No	Wt of test (g)	Amt of std added (mg/ml)	Peak area	Amt recovered (mg)	% recovery
1	0.05823	0.204	305950.81	0.199	97.54 %
2	0.05778	0.204	304576.25	0.202	99.01 %
3	0.05854	0.204	307563.50	0.200	98.03 %
4	0.05902	0.204	308028.18	0.198	97.05 %
5	0.05867	0.204	308484.09	0.201	98.52 %

Table no: 27 Recovery studies for brand II - Ropin

S.No	Wt of test (g)	Amt of std added (mg/ml)	Peak area	Amt recovered (mg)	% recovery
1	0.05498	0.204	305339.98	0.197	96.56 %
2	0.05567	0.204	308225.74	0.196	96.08 %
3	0.05523	0.204	308119.36	0.203	99.50 %
4	0.05492	0.204	306155.43	0.201	98.52 %
5	0.05565	0.204	308951.75	0.199	97.54 %

Chromatogram: 9

Software Version	: 6.3.0.0445	Date	: 12/15/2011
Operator	: manager	Sample Name	: Ropark recovery
Sample Number	: 010	Study	:
AutoSampler	: NONE	Rack/Vial	: 0/0
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 7.50 min
Sampling Rate	: 10.0000 pts/s		
Sample Volume	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 12/15/2011 3:34:18 PM	Cycle	: 12



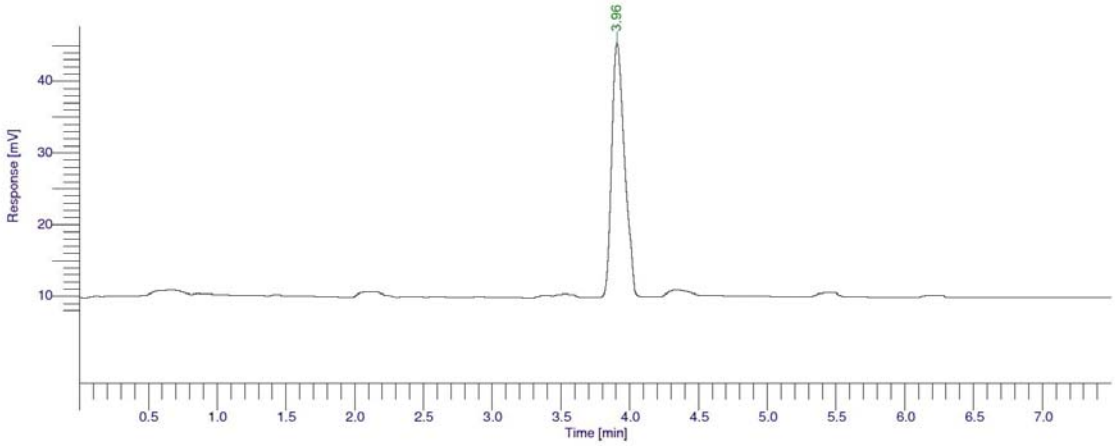
ROPINIROLE HCl

K.M.COLLEGE OF PHARMACY

Peak #	Component Name	Time [min]	Area [uV*sec]	Height [uV]	Area [%]
1		0.691	212.65	136.75	0.06
2		1.499	39.23	19.64	0.01
3		2.264	225.74	143.46	0.06
4		3.256	216.12	141.10	0.06
5		3.932	304576.25	47876.64	99.75
6		4.425	62.16	39.52	0.02
7		5.289	88.62	49.13	0.02
8		6.267	80.46	45.47	0.02
			305501.23	48451.71	100.00

Chromatogram: 10

Software Version	: 6.3.0.0445	Date	: 12/15/2011
Operator	: manager	Sample Name	: Ropin recovery
Sample Number	: 016	Study	:
AutoSampler	: NONE	Rack/Vial	: 0/0
Instrument Name	: HPLC	Channel	: A
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 7.50 min
Sampling Rate	: 10.0000 pts/s		
Sample Volume	: 1.000000 ul	Area Reject	: 0.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 12/15/2011 4:25:12 PM	Cycle	: 18



ROPINIROLE HCl

K.M.COLLEGE OF PHARMACY

Peak #	Component Name	Time [min]	Area [uV*sec]	Height [uV]	Area [%]
1		0.675	280.14	146.78	0.10
2		1.486	38.49	19.22	0.01
3		2.226	312.17	163.92	0.10
4		3.516	68.72	41.76	0.02
5		3.963	308119.36	49986.23	99.57
6		4.334	284.69	147.63	0.10
7		5.494	183.26	112.34	0.08
8		6.256	80.72	52.33	0.02
			309367.55	50670.21	100.00

PARAMETERS USED FOR HPLC METHOD VALIDATION

Number of Theoretical Plates (N)

$$N = 5.54\ T / W$$

The assessment of performance of efficiency of a column is in terms of the number of theoretical plates.

Where,

T - Retention time

W - Width of peak at half height

Tailing Factor

$$T = W / 2f$$

The assessment of peak shape is in terms of symmetry factor.

W - Width of peak at 5 % height

f - Distance from the peak maximum to the leading edge of the peak, the distance being measured at a point 5 % of the peak height from the baseline (or) width of front half of the peak at 5 % height.

Limit of Detection

The detection limit of an individual analytical procedure is the lowest amount of an analyte in a sample which can be detected but not necessarily quantitated as an exact value. Based on the standard deviation of the response and the slope, the detection limit (DL) may be expressed as.

$$LOD = 3.3\ \sigma / S$$

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve (of an analyte)

Limit of Quantitation

The quantitation limit of an analytical procedure is the lowest amount of an analyte in a sample which can be quantitatively determined with suitable precision and accuracy. Based on the standard deviation of the response and the slope, quantitation limit (QL) may be expressed as:

$$\mathbf{LOQ = 10\ \sigma / S}$$

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve (of an analyte) [50]

Precision

The precision study of this method was done by the data obtained from the assay results shown in the table.

Table no: 28 Data for precision

S. No.	Name of tablet	Std deviation	Co-efficient of variation (% RSD)	SEM
1	Ropark	1.180	1.1785	0.5276
2	Ropin	0.9601	0.9441	0.4294

Table no: 29 System suitability parameters

S.No	Parameters	Observed values	Acceptance criteria
1	No. of theoretical plates (N)	2085	Not less than 2000
2	Tailing factor	0.5	Not more than 2
3	Linearity range	4 - 16 µg/ml	-
4	Correlation coefficient	0.9998	0.9999
5	Limit of detection	0.16 µg/ml	-
6	Limit of quantitation	0.48 µg/ml	-
7	% RSD	1.0613	Not more than 2

6. RESULTS AND DISCUSSIONS

Method - I

UV Spectrophotometric Determination of Ropinirole Hydrochloride using 0.1 M Acetic acid

Under the experimental conditions expressed, calibration curve, assay of tablets and recovery studies (accuracy) were performed.

In this proposed method Beer's law was obeyed in the concentration range of 4 - 32 μg/ml.

The percentage recovery was found to be 98.36 % for brand I (Ropark) and 98.16 % for brand II (Ropin).

The result of interference studies showed that the excipients present in the tablet formulations have no effect in the absorbance of drug.

Hence this developed method could be used for routine analysis of ropinirole hydrochloride in bulk drug and its formulations.

Table no: 30 Optical characteristics and statistical data

λ max	250 nm
Beer's law limit (μg/ml)	4 - 32 μg/ml
Correlation coefficient (r)	0.9999
Regression equation* (Y)	0.0313X+0.0014
Slope (b)	0.0313
Intercept (c)	0.0014

* Y = bX + c, where X is the concentration of drug in μg/ml.

Method - II

Extractive Spectrophotometric Estimation of Ropinirole hydrochloride using 0.2 % Picric acid

Under optimum conditions described, the calibration curves were constructed for the drug. Assay of tablets and recovery studies (accuracy), stability of colored species, effect of concentration of picric acid and effect of volume of reagent were performed.

This method was obeyed Beer's law in the concentration range of 2 - 10 μg/ml.

The percentage recovery was found to be 98.55 % for brand I (Ropark) and 98.18 % for brand II (Ropin).

The result of interference studies showed that the excipients present in the tablet formulations have no effect in the absorbance of drug.

Hence this developed method could be used for the routine analysis of ropinirole hydrochloride.

Table no: 31 Optical characteristics and statistical data

λ max	410 nm
Beer's law limit (μg/ml)	2 - 10μg/ml
Correlation coefficient (r)	0.9998
Regression equation* (Y)	0.0274X+0.0013
Slope (b)	0.0274
Intercept (c)	0.0013

* Y = bX + c, where X is the concentration of drug in μg/ml.

Method - III

RP-HPLC Method for the Estimation of Ropinirole hydrochloride

RP-HPLC method was developed as per ICH guidelines. The system suitability parameters proved that the proposed method is suitable for the estimation of ropinirole hydrochloride.

The chromatograms of ropinirole hydrochloride were found to be satisfactory on chromosil $C_{18,}$ 5µm, 250 mm x 4.6 mm, using mobile phase composition of buffer and acetonitrile in the ratio of 70:30. The proposed system of stationary phase and mobile phase was ideally suitable for this estimation as indicated by good number of theoretical plates. The sensitivity of the method was good and also linearity was observed over a concentration range of 4 - 16 µg/ml. The accuracy of the method was determined by recovery studies and it was found to be 98.03 % for brand I (Ropark) and 97.64 % for brand II (Ropin).

Hence this developed RP-HPLC method could be used for routine analysis of ropinirole hydrochloride.

Table no: 32 System suitability parameters

S.No	Parameters	Observed values	Acceptance criteria
1	No. of theoretical plates (N)	2085	Not less than 2000
2	Tailing factor	0.5	Not more than 2
3	Linearity range	4 - 16 µg/ml	-
4	Correlation coefficient	0.9998	0.9999
5	Limit of detection	0.16 µg/ml	-
6	Limit of quantitation	0.48 µg/ml	-
7	% RSD	1.0613	Not more than 2

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